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JUSTIN L. POWERS, EDITOR, WASHINGTON, D. C.

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Penicillin—A Review*

By Walton Van Winkle, Jr., and Robert P. Herwick

HISTORICAL

The discovery of the antibacterial properties of penicillin antedated its clinical use by eleven years. Alexander Fleming, working in the laboratories of the Inoculation Department of St. Mary's Hospital, London, reported in 1929 the discovery of an antibacterial substance produced during the growth of a particular strain of the mold *Penicillium* (1). This paper described the major actions of this substance, which Fleming called "penicillin," against various organisms; noted that the toxicity of crude broths containing this substance was relatively low, and suggested that penicillin might be useful in the treatment of pyogenic infections. Although Fleming was searching for an inhibitor of *Hemophilus influenzae*, his discovery of penicillin was one of those fortuitous laboratory accidents, the significance of which might have been overlooked by a less curious mind. He noted that agar plates which had become contaminated with an air-borne mold showed clear zones surrounding the mold colonies. This observation suggested to him that the mold produced some substance capable of inhibiting the growth of the staphylococci with which the plates had been inoculated. He isolated this mold, grew it on a broth medium, and tested the broth for its antibacterial properties against a variety of microorganisms. This experiment led to the discovery that the substance was active against the pyogenic cocci, certain Gram-positive bacilli, but ineffective against the colon-typhoid group and many other Gram-negative organisms.

Fleming did not pursue his investigations of penicillin and, indeed, little interest appeared to be manifest in his paper. As a result, the American worker Dubos was the first to isolate in clinically usable form an antibacterial agent (2). In 1939, Dubos, working at the Rockefeller Institute for Medical Research, obtained from the autolysates of the soil organism *Bacillus brevis* an extract which selectively inhibited the growth of certain Gram-positive organisms. This substance, tyrothricin, was later found to be composed of two entities, gramicidin and tyrocidin. Unfortunately, tyrothricin was found to have limited usefulness due to its great toxicity upon injection and its lack of activity when taken orally. Its field of usefulness is confined to topical application.

Meanwhile, commencing in 1936, a group of scientists at Oxford University under the leadership of Prof. H. W. Florey commenced a study of the extraction, purification, and

* Received March 12, 1945, from the Food and Drug Administration, Federal Security Agency, Washington, D. C.

clinical usefulness of penicillin. Fleming had tentatively identified the mold as a strain of *Penicillium rubrum*. Thom, in 1932, examined Fleming's mold and identified it as *Penicillium notatum*, Westling, belonging to the *Penicillium chrysogenum* group. It was with this organism that Florey and his associates commenced their epoch-making researches.

After four years of intensive study, the Oxford group published their first report (3), in which they announced the successful concentration of the active material derived from the broth upon which the mold had grown; and showed that this concentrate, in the form of a dry powder, was active *in vitro* against one species each of Streptococcus, Staphylococcus, and Clostridium. They demonstrated that mice could be protected against lethal doses of these organisms by injection of the crude extract of penicillin and that this extract in itself had a low toxicity and relatively few pronounced pharmacodynamic actions.

The following year, in August, 1941, the Oxford group presented the results of their work in great detail and reported the first clinical use of penicillin (4). The first human being to be treated with penicillin was a policeman suffering from a staphylococcus cellulitis of the face and scalp and an osteomyelitis of the humerus. This was a far-advanced case, and at the time treatment was instituted the man was moribund. However, he responded dramatically to penicillin —the lesions on the face improved, the fever subsided, and subjectively the patient felt he was on the road to recovery. Unfortunately there was insufficient material for more than five days' treatment, and when the treatment was ceased, the patient relapsed and died. Nevertheless, the results were so dramatic and the absence of untoward toxic reactions from this crude penicillin injected intravenously so encouraged the workers that further clinical trials were made. In every case dramatic improvement was noted, but because of the small amounts of the drug that could be produced on a laboratory scale, the patients could not receive sufficient material over a long enough period to achieve a clinical cure.

Following the announcement of the Oxford workers of the successful use of penicillin in the treatment of serious pyogenic infections, attention was focused upon the possibility of producing this substance in usable quantities. England was being hard pressed by the war; the United States was preparing for war and entered the hostilities that year. It was quickly recognized that penicillin might be a potent and useful weapon in the fight against infection and disease which must be waged simultaneously with all military engagements. Under the auspices of the Rockefeller Foundation, Professor Florey and his co-worker Heatley came to the United States. They visited the Northern Regional Research Laboratories of the United States Department of Agriculture at Peoria, Illinois, where a group under Dr. Robert D. Coghill embarked on an extensive study of the cultural characteristics of the parent mold, together with studies of purification methods.

Two additional governmental agencies stepped into the picture, realizing that penicillin was a vital military weapon. Under the encouragement of the Committee on Medical Research of the Office of Scientific Research and Development, and the Division of Medical Sciences of the National Research Council, commercial establishments undertook the task of developing methods for the large-scale production of the drug. The War Production Board, through the Drugs and Cosmetic Branch of the Chemicals Bureau and through the Office of Production Research and Development, secured priorities for plant equipment, encouraged commercial plant expansion, and established a system of control and allocation as soon as supplies of penicillin were made available.

The Committee on Medical Research of the Office of Scientific Research and Development devoted its major attention to clinical studies on penicillin designed to show the extent of the drug's usefulness, the dosage, and the duration of treatment, and to gather the other necessary information incident to its intelligent use.

With unbelievable rapidity the large-scale production of penicillin became a reality. The first experimental batches were produced early in 1942, and by the fall of the next year, regular shipments of the drug were being made to the Armed Forces by several firms. The production increased by astronomical increments, and today the Armed Forces receive their full requirements, and nearly all civilians who need the drug can obtain it.

With the advent of commercial production, the Food and Drug Administration entered the picture. New drug applications for penicillin were processed quickly because of the cooperation of the Office of Scientific Research and Development. At the request of the Army, Navy, and War Production Board, a system of pre-distribution control was set up. Research on toxicity, irritation, pyrogens, sterility, stability,

and assay methods was undertaken by the Food and Drug Administration.

ASSAY

Before satisfactory chemotherapeutic investigations could be made, a method for determination of the potency of penicillin and a measure of its activity had to be established. Fleming (1) used a serial dilution method to test the activity of his crude broths. He also employed the "gutter" and "streak" methods and later, the "agar-cup" technique of assay. The Oxford group, however, felt that the serial dilution method was too cumbersome for routine use and proposed the so-called "Oxford Cup-plate Method," which is the forerunner of the present-day "cup-plate" method (4). The Oxford group also defined the "unit" as "that amount of penicillin which when dissolved in 1 cc. of water gives the same inhibition as the standard." This standard was a partially purified solution of purely arbitrary strength.

At the present time there are several methods for assaying penicillin, and these have special fields of usefulness. The assay method used to determine the potency of commercial samples by the Food and Drug Administration is a modification of the original cup-plate method described by the Oxford workers. The details of this procedure may be obtained from the Food and Drug Administration and the method of determining the potency and the error of the assay has been described by Knudsen (5).

The original cup-plate method described by the Oxford group was based on the principle that the diameter of the clear zone surrounding a small open-ended cylinder resting on a seeded agar plate is roughly proportional to the concentration of the penicillin which diffuses from the cylinder, or "cup," into the agar. This method had the disadvantage that the diameter of the clear zone was also affected by other, uncontrollable, variables and, hence, reproducible assays based on the diameter of the zone were difficult and often impossible to obtain. The introduction of a reference standard penicillin, as will be described, compensated for these errors and permitted greater reproducibility of assay values. The statistical methods proposed by Sherwood, Falco, and deBeer (6), by Bliss (7), and by Knudsen (5) permit greater accuracy with this method and provide a measure of the reliability of each assay.

Several modifications of the cup-plate method of assay have been proposed. Vincent and Vincent (8) have proposed to substitute filter paper disks for the cups; Fleming (9) proposed introducing the penicillin solution into a hole cut in the agar on the assay plate. None of these modifications has supplanted to any extent the so-called "cup" technique. Refinements in the assay procedure, such as the use of guides for placing the cylinders on the plates (10), have been made, and these have contributed to the speed and convenience of the method without affecting the accuracy or principle upon which the procedure is based.

Staphylococcus aureus is the organism used most frequently in the assay of penicillin. However, Foster and Woodruff (11) proposed the use of *Bacillus subtilis* because of certain desirable growth characteristics and because of its great sensitivity to penicillin.

The serial dilution method of assay, while too cumbersome and time-consuming for routine assay of penicillin samples, has certain definite fields of usefulness. It, or modifications of it, are used during the manufacture of penicillin as a control procedure, and the method, with some modification, is most useful for assaying the penicillin content of blood or other body fluids. The original serial dilution method, described by Fleming, has been modified by Foster and Woodruff (12). Rammelkamp (13) adapted the method to meet the clinical problem of measuring very low levels of penicillin in blood or other body fluids. The test organism for this method is a Group A strain of hemolytic streptococcus. Subsequent modifications have substituted a highly sensitive strain of *Bacillus subtilis* for the Streptococcus, and it is claimed that this results in an increase in sensitivity and accuracy. Other minor modifications of the serial dilution method have been suggested by Wilson (14). An extremely rapid method utilizing the formation of hemolysin as an indicator has been developed by Rake and Jones (15).

As an outgrowth of the serial dilution method, techniques involving the measurement of the light transmission of broth cultures to which penicillin has been added have been developed. These so-called "turbidimetric" methods have the advantage that the end point is measured electrically and one element of variability is reduced. Use is made of a photoelectric turbidimeter for detecting the end point, and the method is rapid and less cumbersome than the serial dilution method. Foster (16) first described this method, and it has been improved by Foster and Wilker (17), McMahan (18), Holmes and Lockwood (19), and Lee and co-workers (20). It is claimed by Lee that their method will yield reasonably accurate results in ninety minutes.

Heilman (21) has developed a method of assay which does not depend upon comparison with a reference standard penicillin of known potency. A determination is made of the amount of penicillin necessary to prevent the growth of pneumococci in semisolid tissue culture medium. The method has the inherent disadvantage of all tissue culture techniques. Furthermore, it is not suitable for blood or tissue fluid assays since serum interferes with the test.

With the exception of Heilman's method, the assay procedures for penicillin depend upon the comparison of an unknown sample with a standard penicillin of known potency. Before crystalline penicillin became available, impure material which had been compared with the original Oxford solution was distributed by one or two commercial laboratories and later by the Northern Regional Research Laboratory of the Department of Agriculture. Because of the variations in assay procedure and lack of a uniform standard, it became highly desirable to establish a uniform standard and have available sufficient quantities of a reference standard so that all workers could have some basis on which to compare the results of their work. Through the generosity of three of the largest producers[1] of penicillin, a quantity of pure crystalline penicillin sodium was made available to the Food and Drug Administration. This material, after examination for uniformity, was assayed against the standard currently in use by the various laboratories whose activity in terms of "Oxford units" had previously been established, and was set up as a "master standard." A generous quantity of a moderately pure calcium salt of penicillin, donated by one manufacturer,[2] was carefully compared with the "master standard" by a number of collaborating laboratories, and this material set up as a "reference standard." Small quantities of this reference standard are distributed to producers of penicillin on a monthly basis and to others when a definite need for the standard exists.

As a result of the collaborative tests made in establishing this standard, which have been reported by Hunter and Randall (22), the potency of the crystalline" master standard" was fixed at 1650 "units" per milligram, and of the reference standard at 370 "units" per milligram. In order to achieve international uniformity with respect to penicillin potency, an international conference on penicillin was held in London in October, 1944, under the auspices of the Health Organization of the League of Nations (23). At this conference the crystalline penicillin G was adopted as the international standard, and the international unit of penicillin was defined as "the specific penicillin activity contained in 0.6 γ of the International Penicillin Standard." The calcium salt reference standard of the Food and Drug Administration was adopted as the International Penicillin Working Standard, and its activity was defined in relation to the International Penicillin Standard so that 2.7 γ of the International Working Standard contains 1 International Unit of penicillin.

[1] Chas. Pfizer and Co., E. R. Squibb and Sons, and Merck and Co.

[2] Chas. Pfizer and Co.

PRODUCTION

Because of wartime restrictions it is not possible to discuss in detail the methods by which penicillin is now being produced on a commercial scale. However, the basic principles of the various methods now in use may be outlined.

In the laboratory, in early pilot plant operation, and in some commercial establishments, the surface culture technique has been used. This is the oldest and simplest method of penicillin production. The basic principles of this procedure were first described by the Oxford workers (4, 24). These investigators used a modified Czapek-Dox medium consisting of small concentrations of nitrates, phosphates, potassium, magnesium, and iron salts plus glucose. After inoculation of the medium with spores of the mold and incubation for six to seven days, a "mold mat" covered the surface of the broth and yellow drops of penicillin-containing fluid appeared on the surface and diffused into the medium. Wide variations in *p*H occurred during this period, but maximum penicillin production was observed at a *p*H of 7.

This method of surface culture of penicillin was used in the early commercial production of the drug. Flasks or bottles containing from a few hundred to several thousand cubic centimeters of media were inoculated and incubated in suitably designed rooms. For large-scale production, thousands of such bottles must be used to obtain a single batch of crude liquor from which the penicillin is extracted. Through the efforts of Coghill and his group at the Northern Regional Research Laboratory, more productive strains of *Penicillium* were discovered, improvements in the media were made, and more precise conditions for maximum penicillin production were established. One of the outstanding discoveries of this group was the value of corn steep liquor as a

constituent of the medium. Fleming obtained yields of 1 to 2 Oxford units per cubic centimeter in his early experiments; today under improved cultural conditions concentrations of 200 units or more per cubic centimeter in the crude broth are not uncommon.

It can be appreciated that the surface culture technique requires a great amount of space, and, more important, a great amount of labor, to obtain usable amounts of penicillin. The manufacturers, under the encouragement of the Office of Scientific Research and Development and the War Production Board, developed the so-called "deep tank" or submerged culture method of penicillin production. This method involves the use of tanks which may vary in capacity from a few hundred to several thousand gallons. Instead of surface growth, the mold is intimately mixed at all times with the medium in the tanks and large volumes of penicillin-containing broth are produced after a suitable interval of time. For satisfactory growth of the mold and production of penicillin, air or oxygen must be bubbled through the medium and extraordinary precautions must be taken to keep the apparatus and its contents free of contamination. Separation of the mold from the penicillin-containing medium is accomplished by filtration and centrifugation. The majority of penicillin now in use is produced by this method. Certain details of the commercial process have recently been described by McKeen (25).

A third method now in commercial use involves the use of a semisolid medium such as bran which has been soaked in a solution of nutrients. The mold is grown on the surface of the bran, in shallow trays, or is intimately mixed with the bran in revolving drums. The penicillin is removed from the bran by a buffered aqueous extraction process.

A fourth method of production has been proposed by Clifton (26). This is the so-called "trickle" method adapted from the acetic acid industry. No commercial production by this method has as yet been made due to the superiority of the submerged culture technique.

Recently Shwartzman (27) has suggested the addition of cellophane to the fluid media and claims enhanced production of penicillin. The use of cellophane was based upon the observation that young colonies of *Penicillium notatum* in fluid medium tend to develop nearer the side walls of the vessel rather than toward the center of the surface of the medium. The cellophane provides a greatly increased surface without a corresponding increase in volume of the medium. Best results were obtained with large numbers of cellophane strips rather than with a single "bag" as originally employed.

Regardless of the method used to produce the crude penicillin, the product must be purified before it is clinically useful. There are many different processes in use commercially for separation of the active material from the medium on which the mold has grown. In principle, these processes involve separation of the penicillin from the inert and ofttimes toxic impurities by extraction with various water-immiscible solvents such as acetone, amyl acetate, chloroform, etc. Adsorption onto activated charcoal is now employed by many producers as one step in the process. Because of the unstable character of penicillin, particularly in an acid solution and when in an impure state, much of the extraction process must be carried out at reduced temperatures. The final stage of the extraction process involves conversion of the free penicillin to its sodium or calcium salt.

Since penicillin, in solution, is relatively unstable as compared with the drug in the dry state, it is necessary to free the final product of moisture. Penicillin is unstable at elevated temperatures, which necessitates drying at low temperature. Shell-freezing and vacuum drying or other similar methods are used to produce the dry powder of commerce. With the present facilities it is possible to produce a product with less than 1 per cent moisture.

In the early days of laboratory and commercial production, the potency of the final product was quite low. The Oxford group obtained a product assaying about 40–50 units per milligram. This would represent about 3 per cent penicillin. Early commercial production gave products ranging from 100 to 200 units per milligram. Since that time, improvements in mold culture and in the extraction process have resulted in the commercial production of batches assaying as high as 1100–1200 units per milligram, or about 80 per cent pure penicillin. Most manufacturers produce products assaying 350 units per milligram or better.

The rapidity with which improvements in the production of penicillin have been made is due in no small measure to the researches of various groups working under contract with the Office of Production Research and Development of the War Production Board. A group at Pennsylvania State College under the direction of Dr. Frank C. Whitmore has studied the extraction and purification process in detail and has contributed much information and data to the producers, result-

ing in improvements in efficiency of operation, increasing yields and a purer product. A group at the University of Wisconsin under the guidance of Drs. W. H. Peterson and M. J. Johnson has studied the fermentation process and growth requirements of the mold with resultant improvements in this phase of commercial production. The problem of the efficient drying of the final product has been under intensive study by Drs. T. K. Sherwood and F. W. Smith at the Massachusetts Institute of Technology. The development of mutant strains of *Penicillium* and an exhaustive investigation of the genetics of this mold with the view of developing more productive strains have been the problem of Dr. George W. Beadle and his group at Stanford University. Recently another significant advance has been made in penicillin production by the Shell Development Company, who have devised a method of continuous extraction leading to an increased yield of high-purity material with a saving in manpower. This so-called "Shell Development Process" is being installed by several producers and pilot plant production is now under way. This process produces a penicillin having a toxicity approximately half that of penicillin produced by other methods even though the potencies of the two preparations in terms of units per milligram are the same. Too much credit cannot be given to the producers of penicillin for the magnificent job they have done in translating the work of these research groups into actual large-scale production of penicillin.

According to War Production Board figures, approximately four hundred million units of penicillin were produced during the first five months of 1943. The total production for 1943 was approximately 22 billion units; for 1944, 1600 billion units; and in January, 1945, 340 billion units were produced in that month alone. The price of penicillin was $20 per one hundred thousand units in July, 1943. In January, 1945, the average price was approximately $2.40 for the same amount.

PHARMACOLOGY

Aside from toxicological investigations there is a paucity of information on the pharmacology of penicillin. The Oxford group (3) reported some observations on the effects of their crude preparations on circulation and respiration of cats. In general, no well-defined or characteristic effects were produced on either the circulation or respiration. The observations of this group are of limited value because of the impurity of the preparations used and the small amounts of material available.

More recently, Van Dyke (28) has studied certain pharmacological properties of pure crystalline sodium penicillin. He found that penicillin is not a hemolytic agent, and does not alter the oxygen consumption of yeast cells, duck erythrocytes, or slices of rat liver suspended in a solution containing 2.05 mg. (3400 units) per cubic centimeter. High concentrations caused a moderate reduction of the amplitude of the beat of the isolated frog's heart without affecting the rate. This action is reversible. No significant effects were noted on the isolated uterus or ileum of guinea pigs, and the response of these organs to acetylcholine and posterior pituitary extract was not altered.

The absorption and excretion of penicillin has been studied by several workers. Florey and his associates (4) observed the urinary excretion of penicillin in their first clinical trials. They were able to recover about half of the administered penicillin in the urine. A careful study of absorption and excretion has been made by Rammelkamp and Keefer (29) and Rammelkamp and Bradley (30). Intravenous injection of penicillin results in a high initial concentration of the drug in the blood plasma, but the concentration falls rapidly and only traces will be found after 30 to 210 minutes depending on the amount injected. Penicillin is rapidly absorbed when given intramuscularly and slowly absorbed after subcutaneous injection. Urinary excretion is prompt and rapid following intramuscular injection, but is delayed after subcutaneous injection.

Absorption of penicillin from the duodenum is rapid, but oral or rectal doses are very poorly absorbed. This is explained by the observation that penicillin is inactivated at low *p*H (31) and that *Escherichia coli* has an inactivating effect on the substance.

In renal failure, penicillin is very slowly excreted and high blood levels may be maintained for long periods. Rammelkamp and Bradley (30) also observed that simultaneous administration of diodrast partially blocked the excretion of penicillin by the kidneys. This suggests that penicillin is eliminated by way of the tubules. Recently Beyer, *et al.* (32), have shown that para-amino hippuric acid will block the excretion of penicillin, thus permitting higher plasma levels of the antibiotic to be maintained over long periods of time.

Rammelkamp and Keefer (33) have studied the absorption and excretion of penicillin administered intrathecally to normal persons and patients with meningitis. In normal subjects, administration of 5000

to 10,000 units of penicillin was followed by slow absorption and slow urinary excretion, and detectable amounts of penicillin were found in the spinal fluid thirty one and one-half hours after the injection of 10,000 units. The high dose was followed by headache, vomiting, and other signs of meningeal irritation. The low dose produced only a mild headache.

In patients with meningitis, absorption of penicillin administered intrathecally was more rapid than in normal persons. However, in these patients no untoward reactions were noted with doses as high as 10,000 units.

Intraventricular administration has been suggested by McCune and Evans (34), but severe reactions consisting of coma and vascular collapse have been reported by Johnson and Walker (35) following administration of penicillin by this route, and they suggest considerable caution and low doses in this form of therapy.

One property of penicillin which has made it a most striking chemotherapeutic agent is its low toxicity in the doses which are therapeutically effective. Even the early crude preparations seldom gave rise to more than a mild irritation at the site of injection and an occasional urticarial rash or headache (36). Robinson (37) found that the toxic dose of crude penicillin was about 64 times the effective dose in mice as determined by subcutaneous injection.

Using the tissue culture technique, Herrell and Heilman (38) compared the relative cytotoxic powers of a number of common antiseptics with penicillin. Penicillin proved to be considerably less toxic than these other substances and when compared with gramicidin on a weight basis was about one-tenth as toxic.

Welch and his associates (39) studied the toxicity of six salts of penicillin in mice. These salts were relatively pure and five of them were prepared from a single master lot of penicillin. The following order of increasing toxicity was noted when compared on the basis of milliequivalents of the cation used in the preparation of the salt: Na, NH_4, Sr, Ca, Mg, and K. The same relative toxicity was found for the acetates of these cations. The authors conclude that the toxicity of salts of penicillin is primarily due to the cations used in their preparation. This work has been expanded by this group (40) to include a study of seven salts (lithium added to the series) and the previous observations were confirmed. They also found that all seven salts were equally effective against pneumococcus type I infection of mice. Welch and his associates have also made a study of the toxicity of commercial penicillin (41). They found wide variations in the acute toxicity of these products. Some manufacturers' penicillin was lethal for mice at levels of 3500 to 5000 units per 20-Gm. mouse, whereas other manufacturers consistently produced products having a lethal dose of 25,000 to 32,000 units. Part of the observed variability in toxicity appeared to be related to the potency of the product in terms of units per milligram, the purer products having the least toxicity.

CHEMISTRY

Because of wartime restrictions, little can be said of the chemistry of penicillin. It is a relatively small molecule containing carbon, hydrogen, oxygen, nitrogen, and sulfur. It is a strong organic acid. It is inactivated at very low or high *p*H, but is moderately stable at *p*H 6–7. Penicillin is quite soluble in water and alcohol, but is inactivated by the latter agent. It has been produced in crystalline form and its optical properties have been determined. Pure crystalline penicillin is colorless or white, but the article of commerce varies from brownish orange to buff color depending on its purity and the nature of the contaminants.

An exhaustive study of the stability of commercial penicillin has been made by Randall, Welch, and Hunter (42). Stored at 8° C., no significant loss in potency occurred in commercial samples during the course of a year. At 25° C. and at 37° C., some lots remained stable and others lost considerable activity. At 56° C. all samples lost considerable potency. Crystalline penicillin sodium held for one month at 56° C., or heated for two hours at 100° C., showed no detectable loss of activity. The stability of magnesium, lithium, potassium, strontium, calcium, and ammonium salts of penicillin was the same as that of the sodium salt with the exception of the ammonium salt, which lost all potency within three weeks at 56° C.

BACTERIOLOGY

Fleming, and later the Oxford group, demonstrated the principal actions of penicillin with respect to the classes of microorganisms it affects. In general, penicillin is effective against Gram-positive organisms whether aerobic or anaerobic, and relatively feeble in its action against Gram-negative organisms. An exception to the statement with regard to Gram-negative organisms is found in the action of penicillin against the *Neisseria* and other Gram-negative cocci. As a matter of fact, penicillin is the most potent weapon available today against the gonococcus.

Recently, Hobby (43) has shown that penicillin possesses slight but definite activity, *in vitro*, against such Gram-negative organisms as *Eberthella typhosa*, *Shigella dysenteriae* (Flexner), *Proteus vulgaris*, and *Brucella abortus*. This activity is found to be greatest in high potency preparations and has been shown not to be due to the presence of notatin or other by-products of penicillin production.

Penicillin has been shown to have antispirochetal activity, *in vitro* (44). This has been confirmed by Eagle and Musselman (45). Mahoney and his associates (46) have reported effects in rabbit syphilis but Ercoli and Lafferty (47) find that although the treponemes disappear from the lesions promptly and healing takes place in ten to fifteen days after doses of 4000 to 16,000 units/Kg. administered over a period of seventy-two hours, doses as high as 282,000 units/Kg. were not sufficient to sterilize the lymph nodes as tested by transplantation into untreated rabbits. It is probable that until questions of dose, duration, and interval between administration have been evaluated, no clear-cut results in experimental syphilitic infections will be obtained.

Parker and Diefendorf (48) have tested the activity of penicillin against several virus infections. They found it ineffective against vaccinia, St. Louis encephalitis, or equine encephalomyelitis. However, penicillin did have a very definite effect on the course of the disease induced in chick embryos by the viruses of psittacosis and meningopneumonitis. The doses necessary for treatment were quite large, and may not be practicable for human infections.

Penicillin has also been found to possess no activity against a wide variety of yeasts and fungi (49).

The mode of action of penicillin has not as yet been fully elucidated. Hobby and her associates (50) have reported extensive studies on the rate of killing of bacteria by penicillin and the factors which influence this action. They have shown that penicillin is not absorbed or destroyed in the process of bacterial inhibition. Waksman (51) has emphasized the selectivity of action of the antibiotics as compared with ordinary antiseptics and expresses the opinion that antibiotics, including penicillin, are both bacteriostatic and bactericidal. Foster and Wilker (17) present two possible explanations of penicillin inhibition. The first assumes that the bacterial inoculum consists of cells of various sensitivities and that graded doses of penicillin will inhibit cells of correspondingly greater resistance. The second theory assumes all cells to be of the same resistance and the action of penicillin is to prolong logarithmically the generation time of every cell. Based on experiments with staphylococci, Foster inclines to the second theory, but later work by Hobby does not seem to support either theory.

Gardner (52) and Smith and Hay (53) have observed morphological changes in bacterial cells after treatment with penicillin. These changes consist of elongation of the cell and filament formation. These changes can be seen with penicillin concentrations lower than necessary to inhibit completely the growth of the bacteria. Miller, *et al.* (54), have recently shown similar morphological changes in smears from gonorrheal patients treated with penicillin.

Recent work by Rammelkamp and Keefer (55), Miller and Foster (56), Hobby and Dawson (57), and Rantz and Kirby (58) has established the following facts concerning penicillin activity: Pneumococci, gonococci, meningococci, and hemolytic streptococci are very much more sensitive to the action of the drug than are staphylococci. Multiplication of the organism appears to be necessary for the establishment of the penicillin effect. Penicillin appears to produce lysis of susceptible organisms. It is believed that penicillin makes the bacteria unable to complete the normal process of fission. Blood and serum appear to enhance the action of penicillin, and it has been suggested that in the case of meningococci this enhancement is due to a heat-labile component, presumably complement. Enhancement of phagocytosis is not a factor, and Welch, *et al.* (59) have shown that phagocytosis is inhibited only with concentrations of penicillin in excess of those achieved clinically. They suggest that this inhibition may be due to an osmotic pressure effect.

Penicillin may be inhibited or destroyed by certain bacterial products, notably *E. coli*. Lawrence (60) has shown that certain lots of the diastatic enzyme clarase is a potent penicillin inhibitor. Bondi and Dietz (61) have shown that several organisms, including *E. coli*, *B. cereus*, *Sh. dysenteriae*, *Alk. fecalis*, and *B. anthracis* produce a penicillinase. Kirby (62) has extracted a potent inactivator from penicillin-resistant staphylococci, and Liebmann, *et al.* (63), describe the preparation of a standard penicillinase derived from a strain of *B. subtilis*. Recently Randall (64) has discovered a simple chemical which is capable of inactivating penicillin and which is useful also in testing the sterility of penicillin preparations. Details of this method are under wartime restrictions and cannot be described.

CLINICAL USAGE

As has been mentioned, the first reports of the clinical use of penicillin were made by Florey and his co-workers. The limited supply of the drug made it impossible to study the therapeutic efficacy in various infections in an extensive and systematic manner. Production of the drug in the United States soon far surpassed that of any other country and the bulk of the information on clinical usefulness, dosage, and methods of administration has been derived from two sources: the group working under the direction of Dr. Chester Keefer and supplied with penicillin by allocation through the Office of Scientific Research and Development, and the various reports made by the medical staffs of Military and Naval hospitals.

Although isolated reports of the clinical use of penicillin had appeared in the American literature as early as 1941 (50), the limited supplies of the drug and the war emergency necessitated a carefully planned and expertly directed clinical evaluation. This planning and direction were carried out by the Committee on Chemotherapeutic and Other Agents of the Division of Medical Sciences of the National Research Council, and the Committee on Medical Research of the Office of Scientific Research and Development. Under their direction clinical studies were initiated in some twenty civilian institutions which were provided with contracts with the Office of Scientific Research and Development (65).

Although Florey and Florey (66) had reported a small series of cases of various infections in which penicillin had been successfully used both parenterally and by local application, the first report on a large series of cases was that of Keefer, *et al.* (67). This report summarized the observations on 500 cases of various infections. They found the drug most effective in the treatment of staphylococcic, gonococcic, pneumococcic, and hemolytic streptococcic infections. The first report of its military use by the United States Army was made by Lyons (68). The Army first commenced their clinical studies of penicillin at Bushnell General Hospital and later at Halloran General Hospital. As supplies increased and the effectiveness of the drug was demonstrated, its use spread to other Army hospitals. The results in chronically infected gunshot fractures were particularly striking and opened an entirely new phase of military medicine.

In the treatment of staphylococcic infections penicillin is the drug of choice. The sulfonamides, while effective in some cases, do not lead to the dramatic results so frequently observed following adequate doses of penicillin. Among the staphylococcic infections, penicillin has proved effective in acute and chronic osteomyelitis, carbuncles, meningitis, cavernous or lateral sinus thrombosis, pneumonia with empyema, carbuncle of kidney, wound infections and burns, and endocarditis (67, 69–74). Of this group of conditions, the chronic staphylococcic infections responded least satisfactorily. For instance, osteomyelitis usually showed a prompt response with cessation of drainage and healing of sinuses as reported by Lyons (68). However, the majority of the patients relapsed (74). Penicillin is still useful in these cases, however, since the patient is better prepared for surgery.

Streptococcic infections including cellulitis, mastoiditis with its intracranial complications, pneumonia with empyema, puerperal sepsis, peritonitis, and endocarditis—all respond dramatically to penicillin (68–71). Probably the most outstanding field of usefulness of penicillin in infections caused by streptococci is its action in subacute bacterial endocarditis (70, 72, 74, 77). In general, early recognition of the condition, intensive treatment with large doses, and continuation of treatment until the temperature has been normal for several days or even weeks, will result in a high proportion of cures. The older the case and the more extensive the anatomical damage, the lesser the proportion of successes. The use of heparin or other anticoagulant agents with penicillin apparently adds nothing to the treatment (75, 76).

Pneumococcic and meningococcic infections usually respond dramatically to treatment with penicillin (69, 71, 74). In most instances penicillin treatment of pneumococcic pneumonia and uncomplicated meningococcic meningitis has been reserved for those cases not responding to sulfonamide therapy. As supplies of penicillin become more available, it is likely that penicillin will become the drug of choice in pneumococcic pneumonia, although its status in meningococcic meningitis must await further information on penetration of the drug into the cerebrospinal fluid and the possible dangers of its intrathecal use. However, there is little doubt of its effectiveness in those cases classed as sulfonamide resistant.

Penicillin is also of benefit and, in fact, the drug of choice in the treatment of actinomycosis, anthrax, and in serum-sensitive patients with diphtheria (67, 72, 74).

Special mention must be made of the use of penicillin in the treatment of gonorrhea. With sulfathiazole, only 80 to 85 per cent of

the patients with uncomplicated infections become cured. Treatment takes from four to seven days and often longer. In the military forces it presents a real problem because of the loss of large numbers of personnel from active duty. Mahoney and his coworkers (78) were the first to demonstrate the effectiveness of penicillin in this condition. With a total dose of 100,000 units given in divided doses over a twelve- to twenty-four hour period, 96 per cent of cases are cured clinically (79, 80, 81). Often organisms disappear from the discharge in from three to six hours and the purulent discharge changes to a serious one in less than twenty-four hours.

Mahoney, *et al.* (46), first reported the action of penicillin in clinical syphilis. This report evoked widespread interest. Since then he (82) and others (83, 84) have reported more fully their observations on both early and late syphilis. The preliminary results have been very encouraging; organisms disappear from the primary lesion in from fourteen to twenty-four hours, and definite reversal or fall in serologic titer has been reported in a high proportion of cases. Relapses have been noted but as the total dose is increased, the incidence of relapse is markedly decreased. It is as yet too early to make any definite predictions regarding the role of penicillin in the treatment of syphilis, but without doubt this agent has a very definite beneficial effect. At present, the recommended dosage schedule of 1,200,000 units or more given over an eight-day period necessitates hospitalization of the patient, but it is hoped that newer dosage forms or long-acting penicillin will overcome this disadvantage.

Penicillin can be administered intravenously either as repeated injections or as a continuous drip (70, 71). It may be given intramuscularly or as a subcutaneous infusion (71). It has been administered intrathoracically and intra-articularly (72). For rapid effects and to achieve high concentrations of the drug in the blood stream, intravenous administration is the method of choice. Concentrations of 1000–5000 units per cubic centimeter for direct injection through a syringe, or 25–50 units per cubic centimeter for constant intravenous therapy are usually employed. Sterile physiological salt solution or 5 per cent glucose is the usual diluent. Because of the rapidity with which penicillin is excreted, necessitating frequent injections or continuous infusions, a search is being made for vehicles to delay absorption, for methods of oral administration, or for methods of blocking excretion. The concomitant use of para-amino hippuric acid has been proposed as a method of blocking penicillin excretion (32). Recently, high doses of penicillin have been administered orally with appreciable quantities of the administered dose recovered in the urine, indicating absorption of active material (85). Approximately four times as much penicillin must be given orally as by parenteral routes in order to achieve the same blood concentrations. Romansky and Rittman (86) have described a suspension of penicillin in peanut oil and beeswax. Concentrations as high as 300,000 units per cubic centimeter can be made and appreciable blood levels of penicillin are observed for twelve to twenty-four hours after injection of 1 cc. of this mixture. Raiziss (87) has reported that penicillin in peanut oil is an effective agent in experimental infections, and absorption is prolonged to the point that only one injection per day is necessary. Trumper and Hutter (88) have suggested ice applications at the site of injection to prolong absorption.

Local administration of penicillin has been used effectively by Florey and Florey (66) and has recently been found useful by local or parenteral application in infections of the eyes, ears, nose, and throat (89–93). Local application of penicillin is of value in empyema, osteomyelitis, infected soft tissue wounds, and pyogenic skin infections.

The dosage of penicillin must be adjusted to the needs of the patient and the type and severity of the condition being treated. The usual daily dose in severe infections varies from 40,000 to 200,000 units. Where repeated injections are being given, the interval between injections should not be greater than three to four hours, preferably two to three hours. In severe fulminating infections, continuous intravenous or intramuscular infusions may be used. Administration of penicillin should be continued until the temperature of the patient has returned to normal and should be resumed if fever is again observed. In subacute bacterial endocarditis, massive doses of 200,000 to 300,000 units per day should be employed. In gonorrhea, on the other hand, a total of 100,000 units given in five injections three hours apart appears to be adequate. The dosage for syphilis is not yet established, but amounts in excess of 1 million units in 8 days are now being employed.

Bloomfield, *et al.* (94), have recently discussed "penicillin failures." They group the failures into the following categories: (*a*) Cases in which the treatment is too brief or the daily dose too small; (*b*) cases in which penicillin fails unless surgical drainage is also done; and (*c*) overwhelming infection, even with a sensitive strain. They are of the

opinion that the development of "penicillin fastness" probably plays little part in therapeutic failures.

CLINICAL TOXICITY

No serious toxic reactions have been reported in patients receiving large amounts of penicillin. The most disturbing type of reaction occasionally encountered is an allergic reaction resembling serum sickness and characterized by a moderate urticaria (67, 68, 95). Hypersensitivity of the tuberculin type has been observed in persons who have worked with molds but not been exposed to penicillin (96). The cutaneous reaction could be obtained with crystalline penicillin. The same reaction could be observed in persons deliberately sensitized to penicillin (95), and occurred in 5 per cent of individuals without prior contact with penicillin. In some individuals repeated multiple intradermal injections of penicillin caused reactions of the arthus type. Feinberg (97) and Rostenberg and Welch (95) have shown that sensitivity to Penicillium spores does not necessarily extend to penicillin and vice versa.

Irritation following intramuscular injection has been another source of unpleasant reactions, particularly when repeated administration is necessary. Putnam, Welch, and their associates (98) have made a study of the intramuscular irritation of commercial products as well as various salts of penicillin. They found that the irritation produced on intramuscular injection was correlated in some degree with the purity of the product, those with a potency exceeding 700 units per milligram being definitely less irritating than less pure products. However, individual variation was great and many patients complained of pain on injection of physiological salt solution. The buttocks was the preferred site of injection. No advantage was seen in using salts other than the sodium.

Many of the minor toxic effects reported are probably due to associated impurities in commercial penicillin. Febrile reactions may, in some instances, be due to the presence of pyrogenic material. The question of pyrogens in penicillin and their removal has been discussed by Welch, *et al.* (99). At present samples of each batch of penicillin are tested for the presence of pyrogens, for their ability to pass a mouse toxicity test, and for their potency and sterility by the Food and Drug Administration. It is proposed to amend the Federal Food, Drug, and Cosmetic Act to provide for the certification of all batches of penicillin prior to shipment in interstate commerce.

OTHER PENICILLINS AND PHARMACEUTICAL PREPARATIONS

It is now recognized that there are at least three chemically different penicillins, all of which have been isolated in crystalline form. Penicillin G is the article of commerce. Some preparations of penicillin G contain as high as 20 to 25 per cent of penicillin X, also misnamed "allopenicillin." Penicillin F has been produced to some extent in Great Britain but is not an article of commerce in this country. It is alleged to be much less stable than either penicillin G or X.

All three penicillins are potent antibiotic agents. They show slight differences in their activity against certain organisms. Using penicillin G as a standard, penicillin F is about two-thirds as active against *B. subtilis* as against *Staphylococcus aureus* whereas penicillin X is somewhat more active against certain strains of *B. subtilis*. When tested *in vivo* against pneumococci, penicillin X is three to five times as potent as penicillin G. It is also more effective in the treatment of gonorrhea (99).

Various pharmaceutical preparations of penicillin are being prepared and studied. Since penicillin will find a field of usefulness in dentistry, dental cones containing penicillin and a sulfonamide have been prepared to be used in infected tooth sockets. Penicillin ointments are being prepared for use in pyogenic skin disorders and in wounds and on dressings. It is claimed that incorporation of penicillin in lipoid soluble bases will prevent deterioration of the active ingredient over relatively long periods of time. It is likely that other preparations containing penicillin will be developed to meet particular needs as soon as supplies will permit.

OTHER AUTIBIOTICS

Waksman (101) has reviewed the various antibiotic substances known up to 1943. Chemically they are divisible into seven groups:

1. Lipoid bodies
   - pyocyanase
   - clavicin
2. Pigments
   - pyocyanine
   - prodigiosin
   - chrysogenum
   - chlororaphin
   - toxoflavin
   - actinomycin
3. Polypeptides
   - gramicidin
   - tyrocidin
   - lysozyme
   - actinomycetin

4. Sulfur-bearing compounds
   gliotoxin
   penicillin
5. Quinone-like compounds
   citrinin
   penicillic acid
   fumigatin
6. Organic bases
   streptothricin
7. Other agents
   fumigacin

On the basis of toxicity for animals, they fall into the following three groups:

1. Nontoxic or very little toxicity
   penicillin
   citrinin
   pyocyanase
   actinomycetin
2. Fairly toxic
   gramicidin
   tyrocidin
   streptothricin
   gliotoxin
3. Highly toxic
   actinomycin

Of all the antibiotics derived from microorganisms, only two others aside from penicillin have received sufficient attention and have properties such that their usefulness in human infections appears worth demonstrating. Streptothricin, derived from a soil actinomyces, was discovered in 1942 by Waksman and Woodruff (102). This agent appears to be effective against many Gram-negative organisms as well as some Gram-positive ones. Recently it has been shown that streptothricin is active against mycobacteria tuberculosis and leprae *in vitro* (103).

Schatz, Bugie, and Waksman (104) have recently described another antibiotic derived from an actinomycetes, *Streptomyces*. This substance they call streptomycin, and it appears to have the same or slightly greater activity than streptothricin (105). Its acute toxicity is the same or less than that of streptothricin and neither appears to be too toxic for clinical trial. Streptomycin is reported to be active against the mycobacteria *in vitro* (106). Recent unpublished work indicates that streptomycin is to be preferred clinically to streptothricin as being both less toxic and more effective.

SUMMARY

In penicillin we have a potent antibacterial agent which is free from major toxicity and effective against a wide variety of pyogenic cocci and Gram-positive bacilli. It is dramtic in its effects on such conditions as staphylococcic cellulitis and gonorrhea. It offers promise as the only effective weapon against subacute bacterial endocarditis, and it may usher in a new era in the therapy of syphilis.

Its production has been a miracle of industry and Government cooperation, and seemingly insurmountable obstacles have been overcome to supply this drug to our Armed Forces and to desperately ill civilians. Much of the story remains to be told.

There is hope that the development of new antibiotics will find other infections, now unresponsive to penicillin, brought under control. A new era in medicine has begun.

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The Stability of Penicillin Sodium Held at Various Temperatures*

By William A. Randall, Henry Welch and Albert C. Hunter

In September, 1943, the Food and Drug Administration undertook the testing of each lot of penicillin produced in this country to determine whether it met the specifications which had been prepared by the Army and Navy. These specifications made it necessary to test the product for potency, toxicity, pyrogenicity, sterility and moisture content. During the early commercial production of penicillin, an expiration date of three months when kept below 10° C. seemed advisable. This made necessary the prompt dispatch of the penicillin to areas where it was needed and created an acute transportation problem where large quantities were needed overseas. One of the first problems, therefore, was to study the stability of penicillin with a view to increasing the effective dating period of the product. On the basis of the data to be presented in this paper and on information submitted by the various manufacturers of penicillin, the dating period of some manufacturers' products has now been increased to twelve months at temperatures below 10° C.

EXPERIMENTAL

The study on stability was initiated by obtaining 10 ampuls of a single lot of penicillin from each of nine different companies. One ampul was used for a base assay and the remainder placed, three each, at 8° C., 25° C. and 37° C. The 37° C. temperature was used to simulate a tropical climate. All of the ampuls were labeled to contain 5000 units with the exception of that from one manufacturer who submitted ampuls containing 25,000 units. The small number of ampuls used was necessary because of the extreme scarcity of penicillin at that time. Later, larger samples consisting of 17 ampuls containing 100,000 units were submitted by 16 manufacturers. These latter lots were placed at 8° C., 25° C., 37° C. and 56° C. The temperature of 56° C. was included because it was felt that deterioration would be accelerated and might give an early clue to the stability at lower temperatures. Except where indicated, all samples were contained in flame-sealed all-glass ampuls.

The data obtained in this study are summarized in Tables I to IV. The numbers in the tables represent percentage loss in potency at the stated time and temperature. The percentage loss in potency represents the combined behavior of all lots tested at the stated time and temperature in order to present an over-all picture of the stability of each manufacturer's product and in some cases is the average for a lot which lost all or nearly all activity with a lot which remained quite stable. This in part accounts for the inconsistencies in the potency lost at various temperatures.

The cylinder plate method was used in the assay procedure and in interpreting the results of this study, allowance should be made for the error known to be inherent in this method. Another source of error is the variation from ampul to ampul in the same lot of penicillin where potency tests are based on the total contents of the ampul rather than on units per milligram.

Stability at 8° C.—Table I shows the stability of penicillin sodium held at 8° C. It can be seen that when due allowance is made for ampul and assay variation, very little loss of potency occurs at this temperature. In the case of manufacturer E, marked deterioration occurred in the aluminum-sealed container while practically no potency was lost in the hermetically sealed ampul. Here we cannot say whether this is ampul variation or actual loss. Marked deterioration of penicillin in aluminum-sealed rubber-stoppered bottles has been noted by us in the case of several manufacturers' products and also in the preparation of penicillin in our own pilot plant. This most often was due to a failure to dry the rubber stoppers thoroughly. This early difficulty has now been almost entirely eliminated but great care must still be taken to ensure dry stoppers and a good seal. Attention is drawn to manufacturer H whose product was quite stable at 8° C. but lost potency very rapidly at higher temperatures. This loss at higher temperatures is shown in the subsequent tables.

TABLE I.—PER CENT LOSS OF POTENCY OF PENICILLIN SODIUM AT 8° C.

MFR.	MONTHS OF STORAGE											REMARKS
	1	2	3	4	5	6	7	8	9	10	12	
A		0		17				38	0			
B						0						AL. SEAL
C						0						
D					7							
E		35 5		51								AL. SEAL GLASS
F				0			10					
G												
H	0	10	12						5			
I			15	4		15				8		
J						10						
K												
L	0		0	0					0		0	
M			4					0			0	
N				18		0	18				20	
O						12						
P			17						0		0	
Q						0						

Storage at 25° C.—Table II gives the results of storage at 25° C. With certain exceptions most manufacturers' products show good stability. In the case of producers E and K, the loss is no

* Received Jan. 12, 1945, from the Food and Drug Administration, Federal Security Agency, Washington, D. C.

doubt due to faulty packaging in the aluminum-sealed container. The product of producer H, however, was contained in all-glass ampuls. In all, six different lots of producer H's penicillin were tested. Two of these lots were unstable at 25° C., one lot losing all potency within one month and the other losing potency in an erratic fashion; one ampul lost 27% in one month, a second ampul lost 80% in two months, and a third lost 19% in three months.

TABLE II.—PER CENT LOSS OF POTENCY OF PENICILLIN SODIUM AT 25° C.

MFR	MONTHS OF STORAGE 1	2	3	4	5	6	7	8	9	10	12	Remarks
A	0		29			53	56					
B		0			12							AL. SEAL
C			0			0						
D			7									
E		80 19										AL. SEAL GLASS
F		0	0			10						
G												
H	49	49	40									
I		16	8		24							
J												
K	71		0									AL. SEAL
L	0	0	0			36					10	
M	0	0	12		23			0				
N	23		33			0						
O		0		10		21						AL. SEAL
P	10		17								8	
Q	0	0				0						

TABLE III.—PER CENT LOSS OF POTENCY OF PENICILLIN SODIUM AT 37° C.

MFR	MONTHS OF STORAGE 1/2	1	2	3	4	5	6	7	8	9	10	Remarks
A	8	6	16			36						
B			19			15						AL. SEAL
C			5	7			10					
D				31	39							
E	100		100 46									AL. SEAL GLASS
F	0	11	5			37	12					
G												
H	90	48	72									
I	0	0	19		40	8						
J			15									
K		38										AL. SEAL
L	0	5	7			11	0					
M	9	10	29		8	40						
N	15	7	13			23						
O	0	32										
P	18	0										
Q	0	0					0					

Storage at 37° C.—At 37° C. (Table III) the accelerating effect of the higher temperature on the rate of deterioration is apparent. This effect is by no means uniform as the product of manufacturers C, F, L and Q showed essentially no loss of potency in six months. It is thus apparent that it is possible to produce penicillin which remains stable for considerable periods at 37° C. The reason for this superior stability of one producer's product over others is not known. It is known that commercial penicillin is not composed of a single chemical compound but may be a mixture of closely related entities which may vary in their stabilities. The kind of impurities present also may affect the stability. Although many lots are as stable at 37° C. as at 8° C., when an unstable lot is encountered, it usually loses potency very rapidly at the higher temperature while retaining its potency quite well in the refrigerator. This is shown especially in the case of the product of manufacturer H.

Storage at 56° C.—In Table IV the results of storage at 56° C. are given. With the exception of producer G, whose material has been tested for a short time only, all of the products suffered losses in potency, from 5% to 72% in two weeks and from 45% to 100% in four months. By contrast, crystalline penicillin sodium stored for one month at 56° C. showed no detectable loss of activity. Furthermore, crystalline penicillin sodium "G" when heated at 100° C. for two hours shows no loss of potency.

Containers.—The all-glass container which the great majority of manufacturers initially used to package their material was found objectionable when used in clinical practice. The glass ampul must be filed, opened, a diluent added, and the contents then transferred to another container suitable for the withdrawal of the solution at various intervals. The bother entailed by this procedure made desirable the packaging of the penicillin in a container with a rubber diaphram so the diluent could be added and the solution withdrawn as desired without the necessity of transfer.

TABLE IV.—PER CENT LOSS OF POTENCY OF PENICILLIN SODIUM AT 56° C.

MFR	MONTHS OF STORAGE 1/2	1	2	3	4	5	6	7	8	9	10	Remarks
A	44	32		66	46							
B	17	55	32	95			72					AL. SEAL
C		58 19	70 19	77 8								AL. SEAL GLASS
D	12	31			72							
E	72		100									
F	0	27	47	61	60							
G	0	0										
H	66	55		90	96							
I	16	1		19	50							
J	59		90*									
K	77	95										AL. SEAL
L	63	36		75	100							
M	55	38		77	90							
N	5	15		38	45							

It was generally agreed that a rubber-stoppered vial with an aluminum seal would most likely be the most suitable for maintaining the potency of the penicillin. Since very little information was available as to the stability of penicillin in this type of container and since very few manufactur-

ers were using an aluminum seal, it was necessary to study the suitability of this closure on penicillin sodium prepared in our own pilot plant. *Penicillium notatum* was grown in surface culture and the crude broth extracted and concentrated by the carbon process. The concentrate was then frozen in a CO_2-alcohol mixture at a temperature of −70° C. The frozen material was then dried on a cryochem apparatus under a vacuum below 300 microns for sixteen hours followed by application of heat (37° C.) for from two to six hours. After drying and while still under vacuum, the vials were clamped off, removed from the cryochem and transferred to a specially constructed dry chamber. Rubber stoppers which had dried for six days in a vacuum desiccator over P_2O_5 were then placed in the dry chamber. The articles in the chamber were manipulated by means of rubber gloves sealed into two openings of the box. In this dry atmosphere, the clamps were removed from the vials, the vacuum replaced by addition of dry nitrogen and the rubber stoppers put in place. The stoppered vials were then removed from the dry box and the aluminum seal attached with a sealing machine. Samples were taken from moisture determination and assay and the remainder of the vials placed at 8° C., 25° C., 37° C. and 56° C.

Potency and Moisture Determinations.—In Fig. 1 are given the potency and moisture determinations at various time intervals, extending over a period of eight months. The penicillin produced in our pilot plant was an extremely hygroscopic material which absorbed moisture very rapidly and completely lost all activity when exposed to the atmosphere at room temperatre. This experiment, therefore, is considered to represent the behavior of a product extremely sensitive to the deleterious effect of moisture.

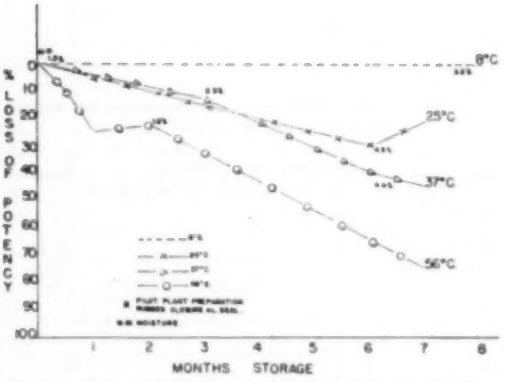

Fig. 1.—Loss of Potency of Penicillin Sodium at Various Temperatures.

The moisture and potency determinations given in Fig. 1 are based on an average of five vials. At the start of the experiment the average moisture content was 1.2% and the potency was 254 units per milligram. At 56° C. in two months the moisture content remained constant while the potency declined approximately 20%. This is comparable to the results obtained with the commercial product in all-glass ampuls which also lost some potency at 56° C. in two months, irrespective of moisture content. Although no moisture determination was made after seven months at 56° C. when the potency loss was 75%, it is reasonable to assume that the uptake was as great as at 37° C. in six months. We believe, however, that at this elevated temperature loss of activity would have occurred even if no additional moisture was absorbed. When we speak of "moisture" we are well aware of the controversial aspects of the use of this term in speaking of penicillin in rubber-stoppered aluminum-sealed containers. Various opinions have been expressed to explain the gain in weight of penicillin in aluminum-sealed vials. The simplest explanation is that moisture is absorbed through the rubber closure. Another is the absorption of either moisture or other material from the rubber stopper. Still a third theory is that a portion of the impure penicillin volatilizes during the moisture determination.

At 37° C. and 25° C. loss of activity was accompanied by an increase in moisture. Moisture uptake up to 2.5% did not result in much if any loss in potency in three months while at six months, when the moisture content reached above 4%, activity had declined 30 to 40%.

In the table only average figures are recorded. The following example shows the variation in moisture content and potency in the five vials used in the determination at six months and 25° C.

	Vial No.				
	I	II	III	IV	V
Moisture, %	4.6	6.5	3.6	4	3.7
Loss of potency, %	40	84	0	16	4

It can be seen that when the moisture rises much above 4%, marked loss of potency occurs. In all cases portions from the same vial were removed for moisture determination and assay.

Of significance is the fact that at 8° C. no loss of potency occurred even though the moisture increased to an average of 3.3%. The moisture of the individual vials was as follows: 4.6, 3.3, 2.2 and 3.1%. It would seem, therefore, that uptake of some moisture at low temperatures is not accompanied by loss of activity. Any study of the stability of penicillin is necessarily a progress report and some phases of this work are still continuing.

Since most of the penicillin produced is put up in rubber-stoppered aluminum-sealed vials, the desirability of storage at temperature below 10° C. is emphasized although short exposures at higher temperatures should have no deleterious effects.

Cation Influence on Stability.—There has been a persistent opinion expressed by many individuals working with penicillin that the calcium salt is more stable than the sodium salt. In order to study the influence of the cation on the stability of penicillin, the ammonium, calcium, lithium, potassium, sodium and strontium salts were prepared by a commercial producer of penicillin.[1] The salts were put up in all-glass ampuls and all had a potency of from 900 u./mg. to 1000 u./mg. Seventeen ampuls of each salt were used in the experiment. One ampul was used for base assay and the others distributed equally among the four temperatures used in previous experiments.

In Fig. 2 is given the potency loss at 56° C. at various time intervals during a four-month period and also the percentage moisture. The ammonium salt lost all potency within three weeks. This may be accounted for by the acidity of the product, which was *p*H 3.5 when brought into solution. The moisture content was 2.33% and the combined effect of the moisture, *p*H, and heat was sufficient to destroy all activity. Potency determinations at other temperatures show a 32% loss at 37° C. and little or no loss at 25° C. and 8° C. This experiment shows

[1] These salts were prepared by Chas. Pfizer & Co. at the request of the Office of Scientific Research and Development.

strikingly the effect of a high temperature on a salt of penicillin having a low *p*H. When the ammonium salt was brought into solution it was partly insoluble until the solution was adjusted to *p*H 6.5.

The remaining salts show an obvious trend downward and in two months lost about 50% of their original potency. The apparent upswing at three to four months may represent assay variation or ampul difference. Since only one ampul was available for testing at each time period, ampul variation could not be tested.

So few potency tests have been made at the other temperatures that these results have not been recorded. What determinations have been made do not reveal any significant difference between the various salts. Of some interest is the behavior of the magnesium salt, of which there were insufficient ampuls for testing at 56° C. This salt had 5.28% moisture. In four months at 37° C. it lost 33% of its original activity and practically none at 25° C. and 8° C. So far, with the exception of the ammonium salt at 56° C., the nature of the cation seems to have no effect on the stability of penicillin.

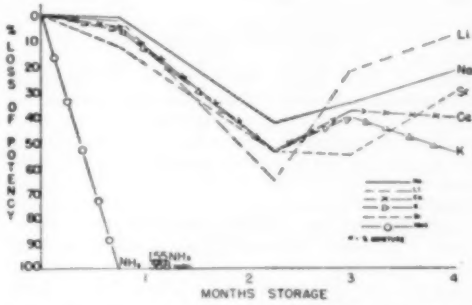

Fig. 2.—Loss of Potency of Six Different Salts of Penicillin at 56° C.

Low Potency Penicillin.—Recently, a few lots of penicillin produced by several manufacturers have been found to be below the figure of 85,000 units for a 100,000-unit ampul, which is the minimum potency required by the Army specifications. In order to explain the low assay value, additional samples of these low potency lots were obtained and assayed in units per milligram. It was found that the units per milligram was markedly lower than that stated by the manufacturer. Our findings were confirmed by the companies concerned and it was generally agreed that the lots involved were examples of unstable penicillin. It was further thought that since the low potency samples coincided with the advent of warm weather, the penicillin may have deteriorated in transit.

In order to ascertain whether the production of unstable lots of penicillin extended to other producers, five ampuls of current production were obtained from each of 18 manufacturers and stored for one month at 37° C. Assays were made at weekly intervals to detect incipient loss of activity. All except two of the producers shipped the material in cartons containing dry ice. Eight manufacturers used aluminum-sealed containers. So far the penicillin of 17 of the 18 manufacturers has been stored for one month at 37° C. No significant loss of potency occurred. It thus appears that the unstable lots encountered were just chance occurrences and did not represent a general trend. The results further demonstrate the general improvement in the stability of penicillin, especially in aluminum-sealed containers.

SUMMARY

The stability of commercial penicillin sodium packaged in flame-sealed glass ampuls and stored at 8° C., 25° C., 37° C. and 56° C. has been followed during the course of a year. At 8° C. no significant loss of potency occurred. At 25° C. and 37° C. some lots remained stable while others lost considerable activity. At 56° C. all samples lost considerable potency. Crystalline penicillin sodium held for one month at 56° C. or heated for two hours at 100° C. shows no detectable loss of activity.

Penicillin sodium of our own manufacture was put up in rubber-capped bottles with an aluminum seal and stored at various temperatures. No loss of potency occurred at 8° C. in eight months, even though there was some increase in moisture content. At the other temperatures the potency gradually declined. The loss was greatest at 56° C., less so at 37° C., and still less at 25° C. This loss of activity was accompanied by an increase in moisture content. When the moisture exceeded 4%, very marked deterioration was noticed.

The stability of the magnesium, lithium, potassium, strontium, calcium and ammonium salts of penicillin in flame-sealed glass ampuls was studied. With the exception of the ammonium salt, which lost all potency within three weeks at 56° C., these salts behaved in the same manner as the sodium salt.

Penicillin of recent manufacture from 17 different producers was found to remain stable for one month at 37° C.

In general these studies indicate that penicillin sodium is a relatively stable product when it is kept below 10° C., and when properly prepared will maintain its potency for at least one year under such temperature conditions. Even at higher temperatures short periods of exposure should have no deleterious effect on the potency.

The Thermostability of Pyrogens and Their Removal from Penicillin*,†

By Henry Welch, Clifford W. Price, Velma L. Chandler, and Albert C. Hunter

Since the original studies of fever-producing substances by Seibert, *et al.* (1, 2, 3) it has been recognized that the pyrogenic substances found in improperly prepared intravenous solutions are relatively thermostable. However, previous to the studies made by these authors, Hort and Penfold (4) had demonstrated that neither boiling nor autoclaving intravenous solutions containing pyrogenic substances would suffice for their destruction. Banks (5) in a carefully controlled study showed that sterilization at 20 pounds' pressure for five hours in an alkaline medium (*p*H 8.2) and two hours' sterilization at this pressure in acid medium (*p*H 3.8) would eliminate the thermogenic substance from solutions. Under ordinary commercial conditions the employment of such elaborate precautions for the removal of pyrogens from solutions through heat treatment is impractical. In answer to this problem, Co Tui (6) and his co-workers developed a method for removal of pyrogens from infusion fluids by filtration with an adsorption filter (Seitz serum pad No. 3) and more recently Co Tui and Wright (7) describe in detail methods for the preparation of nonpyrogenic infusion and other intravenous fluids through the use of adsorptive filtration with Ertel compressed asbestos pads. As pointed out by these authors, removal of the pyrogen is not brought about by sieving but by adsorption and therefore the retentive powers of the filter can be exhausted. Studies in these laboratories confirm the work of Co Tui and his co-workers and demonstrate that when properly prepared, intravenous fluids can be made free from pyrogens without too great difficulty. Our experience, however, in the examination of great numbers of commercially prepared fluids leads us to the conclusion that although the fluids are in most instances prepared free from pyrogenic substances, they are in many cases filled into ampuls which because of improper cleansing contain pyrogens on their surfaces. Thus in the examination of 10 ampuls from a single lot, 2 may be found to contain thermogenic substances, while 8 ampuls do not. In such a case the whole shipment of ampuls would be condemned.

Preliminary studies confirmed the thermostable characteristics of the pyrogen used. Neither boiling nor autoclaving at 120° C. reduced materially the thermogenic properties of the solutions tested. The following studies were then carried out to determine the effect of dry heat on our stock pyrogen material. Method of preparation of this pyrogen (made from Pseudomonas) has been described elsewhere (8).

EXPERIMENTAL

Effect of Dry Heat on Pyrogen Made from Pseudomonas.—A series of ampuls were treated by pouring into them 10 cc. of pyrogen solution and then immediately pouring the material out, thus leaving a film of pyrogen on the inner surface. The treated ampuls were dried at 37° C. for twenty-four hours and heated in an accurately controlled muffle furnace for thirty minutes at varying temperatures. After the heat treatment the ampuls were rinsed with 10 cc. of pyrogen-free water and the rinsings tested by the injection of rabbits with 3 cc. per Kg. of body weight. In order to economize on animals and since we were interested primarily in complete destruction of the pyrogen in the ampuls, three rabbits were injected for testing purposes rather than five as called for in the U. S. P. method. A similar series of ampuls was prepared and treated in an identical manner but in this case 0.5 cc. of the pyrogen solution was added to each ampul and the water removed by lyophilization. The results obtained are given in Table I where it will be noted that a temperature of 250° C. for thirty minutes is necessary to destroy the pyrogen present as a film on the inner surface of ampuls while the lyophilized material was destroyed at 200° C. in thirty minutes. On the basis that a 0.6° C. rise would be considered pyrogenic it would appear from Table I that a temperature of 200° C. for forty-five minutes is sufficient to destroy pyrogen. However, two of the three rabbits injected with pyrogen so treated showed temperature rises of 0.6° C. while the third showed a rise of 0.3° C. only and thus the average rise was 0.5° C.

Since pyrogens are apparently the autolytic products of microorganisms it would be expected that penicillin broth would contain such substances. Inoculation of sterile crude penicillin broth into rabbits was found to produce invariably a typical pyrogenic response. On the theory that the pyrogen present in penicillin was a result of lysis of the mold itself a series of experiments was conducted using mold mats from surface culture, mats from deep tank, dry mycelia from deep tank cultures, and dry bran cultures, to determine the potency of the pyrogen present and its thermostability. All samples were from commercial manufacture except the surface culture mats.

Effect of Dry Heat on Pyrogens from Penicillium Mats.—In the preparation of the pyrogen from the various penicillium mats the following procedure was used:

Seventy grams of dry bran culture was weighed into the receptacle of a Waring Blendor and 450

* Received Oct. 3, 1944, from the Division of Bacteriology, Food and Drug Administration, Washington, D. C.

† By "Penicillin" is meant the product as it is now produced commercially containing varying amounts of impurities.

cc. of pyrogen-free distilled water added. The mixture was blended for ten minutes, centrifuged at high speed (4000 r. p. m. for thirty minutes) and then filtered through a Berkefeld filter which had been heated previously at 250° C. for forty minutes. Seventy grams of the dry mycelium from deep tank culture was similarly treated. The moist surface culture mats and the moist deep tank culture (grayish rather rubbery mass) were similarly treated but in the preparation of pyrogen from these materials 140 Gm. was used.

After preparation suitable dilutions were made with pyrogen-free water and these were tested in rabbits for their ability to produce a thermogenic response when injected in 1.0-cc. amounts. The results are given in Table II where it will be noted that all types of penicillium growth produced a thermogenic response in rabbits in at least a 1:10 dilution while in the case of the dry mycelium obtained from deep tank cultures a pyrogenic response was obtained in the 1:300 dilution. Intravenous injection of mice with these various filtrates of lysed mycelia resulted in death when 0.5 cc. was injected, while as little as 0.1 cc. produced marked toxic symptoms.

In attempts to purify the pyrogen produced from the mycelia of *Penicillium notatum* using a chemical method previously described (8), it appeared that these pyrogenic substances differed somewhat chemically from the pyrogen produced from Pseudomonas. Accordingly, tests were made to determine the thermostability of each of the mycelial growths described above. In these tests 0.5 cc. of pyrogen solution was lyophilized in ampuls and treated in a muffle furnace at varying temperatures, following which pyrogen-free water was added and the solution thus obtained tested in rabbits for its thermogenic effect. The results are given in Table III, where it will be noted that pyrogen produced from surface culture or deep tank culture mats was destroyed at 200° C. for thirty minutes, while both the pyrogens produced from dry bran cultures or dry mycelia (from deep tank culture) were destroyed at a temperature of 250° C. for thirty minutes.

Removal of Pyrogen from Penicillin by Filtration.—In some preliminary filtration experiments it was demonstrated that the Seitz E. K. bacteriological pad had the ability to remove pyrogens from solutions of penicillin containing them. However, the ability of a pad to remove pyrogen was directly related to the size of the pad and the concentration

TABLE I.—EFFECT OF DRY HEAT ON PYROGEN[a]

Ampul No.	No. of Rabbits Injected	Heated in Muffle Furnace to	Time (in Min.) of Heat Treatment	Av. min. (Control) Temperature, ° C., in Rabbits before Injection	Av. Max. Temperature, ° C., in Rabbits after Injection	Av. Rise Temperature, ° C.[b]
1-3	3	150° C.	30	39.5	40.6	1.1
4-9	6	200° C.	30	39.4	40.5	1.1
10-12	3	200° C.	45	39.3	39.8	0.5[c]
13-15	3	250° C.	15	39.3	40.2	0.9
16-21	6	250° C.	30	39.6	39.8	0.2
			Lyophilized Pyrogen			
22-24	3	100° C.	30	39.4	40.9	1.5
25-27	3	200° C.	30	39.3	39.7	0.4
28-30	3	250° C.	30	39.4	39.7	0.3
Control	4	(Unheated film)		39.3	41.2	1.9
Control	3	(Unheated lyophilized material)		39.4	41.4	2.0

[a] Film on Ampul Surface.
[b] Where the average rise in temperature is under 0.6 of a degree none of the rabbits injected showed an average temperature rise of 0.6° except where otherwise indicated.
[c] Two of three rabbits tested showed rises of 0.6° C.

TABLE II.—POTENCY OF PYROGENS PRODUCED FROM *Penicillium notatum*

Type of Penicillium Growth	Dilution Tested	No. of Rabbits Injected	Av. Min. (Control) Temperature, ° C., in Rabbits before Injection	Av. Max. Temperature ° C. in Rabbits after Injection	Av. Rise in Temperature, ° C.[a]
Dry bran culture	Undiluted	3	39.6	41.3	1.7
	1:10	3	39.6	41.1	1.5
	1:100	3	39.3	39.7	0.4
Dry mycelium from deep tank culture	Undiluted	3	39.3	41.8	2.5
	1:10	3	39.2	41.4	2.2
	1:100	3	39.2	40.2	1.0
	1:200	3	39.2	40.2	1.0
	1:300	3	39.3	40.2	0.9
	1:400	3	39.3	39.8	0.5[b]
Deep tank culture mat	Undiluted	3	39.2	40.9	1.7
	1:10	3	39.3	40.6	1.3
	1:100	3	39.3	39.5	0.2
Surface culture mat	Undiluted	3	39.4	40.6	1.2
	1:10	3	39.2	40.3	1.1
	1:100	3	39.1	39.5	0.4

[a] Where the average rise in temperature is under 0.6 of a degree none of the rabbits injected showed an average temperature rise of 0.6° except where otherwise indicated.
[b] One of the three rabbits tested showed a rise of 0.9° C.; the other two rabbits a rise of 0.2° C. and 0.5° C., respectively.

of the pyrogen in the penicillin. As pointed out by Co Tui, *loc. cit.*, the retentive properties of the filter can be exhausted by overloading it with solutions containing pryogenic material. It was found that with the so-called standard pyrogen (pyrogen prepared from Pseudomonas cultures) the thermogenic agent could be removed from 50 cc. of 1:1000 dilution by filtration through a double pad (small size Seitz 1-inch pad). A single pad failed to remove all the pyrogen from 50 cc. of solution containing this dilution of pyrogen. The 1:1000 dilution of our standard pyrogen contains approximately eight times the amount of thermogenic substance necessary to produce a positive Pharmacopœial test. A 1:100 dilution of our standard pyrogen required five pads (1-inch E. K. Seitz pad, 30-cc. capacity filter) to reduce the thermogenic substance in 50 cc. of solution so that it no longer gave a positive Pharmacopœial test, but even with five pads there was some indication in the rabbits injected with the filtered material of the presence of thermogenic substances. Attempts were made also to filter a 1:10 dilution of the standard pyrogen (Seitz 1-inch pad) with an increasing number of pads without success. In this case we were limited in the number of pads used by the pad capacity of the filter.

In order to determine the capacity of a 3-inch Seitz pad, a solution was prepared containing a 1:1000 dilution of our standard pyrogen and filtered in 250-cc. increments. Each increment was then tested in a series of 3 rabbits. A large number of such experiments indicated that 1250 cc. of distilled water containing a 1:1000 dilution of pyrogen could be filtered free from pyrogen by means of a 3-inch Seitz pad. The next increment (1250–1500) on injection into 3 rabbits showed an average rise in temperature of 1.13° C. In order to determine the ability of this type of pad to remove pyrogen from pencillin solutions several lots of penicillin produced by seven different manufacturers were tested. The penicillin was diluted to contain 5 units per cc. To each of these solutions was then added a 1:1000 dilution of our standard pyrogen and the pyrogen-penicillin combinations filtered through a 3-inch Seitz pad in 500-cc. increments. Each 500-cc. amount was tested for the presence of thermogenic substances by the injection of three rabbits. The first 500-cc. increment to come through the filter containing pyrogenic substances which would cause an average rise in temperature of the three rabbits of 0.6° C. or more was considered to be the end point (capacity of the filter for that lot of penicillin).

The results of these studies are given in Table IV where it is shown that there is a marked difference in the ability of the filter pad to remove pyrogens from the penicillin produced by different manufacturers. There appears to be little or no loss of penicillin in the filtration process. A study of Table IV will show that distilled water containing 1:1000 dilution of standard pyrogen was filtered free of pyrogen up to 1250 cc. of solution, while the next increment (250 cc.) was found to be pyrogen-positive. On the other hand, it will be noted that the penicillin of manufacturers A, B, C and D, containing the same amount of standard pyrogen, was successfully filtered free from pyrogen up to 2 L. and that it was not until the next 500 cc. was put through the filter that a thermogenic response was obtained in rabbits with the filtrate. In the case of the products of manufacturers E, F and G, as much as 3 L. of the product of manufacturer E and 4 L. of the

TABLE III.—EFFECT OF DRY HEAT ON PYROGENS PRODUCED FROM *Penicillium notatum* CULTURES

Ampul No.	Type of Penicillium Growth Tested	Number of Rabbits Injected	Heated in Muffle Furnace to	Time (in Min.) of Heat Treatment	Av. Min. (Control) Temperature, ° C., in Rabbits before Injection	Av. Max. Temperature, ° C., in Rabbits after Injection	Av. Rise in Temperature, ° C.
1	Dry bran culture	1	(Unheated control)		39.1	40.5	1.4
2–4		3	150° C.	30	39.3	40.9	1.6
5–7		3	200° C.	30	39.1	39.6	0.5
8–10		3	250° C.	30	39.5	39.6	0.1
11	Dry mycelium from deep tank culture	1	(Unheated control)		38.8	41.6	2.8
12–14		3	150° C.	30	39.0	40.0	1.0
15–16		3	200° C.	30	39.3	40.0	0.7
17–19		3	250° C.	30	39.3	39.7	0.4
20	Deep tank culture mat	1	(Unheated control)		39.0	40.0	1.0
21–23		3	150° C.	30	39.3	39.8	0.5
24–26		3	200° C.	30	39.6	40.0	0.4
27	Surface culture mat	1	(Unheated control)		39.3	40.7	1.4
28–30		3	150° C.	30	39.3	39.9	0.6
31–33		3	200° C.	30	39.2	39.5	0.3

TABLE IV.—FILTRATION OF PENICILLIN CONTAINING PYROGEN

Manufacturer	Penicillin U/Cc.[a] before Filtration	Penicillin U/Cc.[a] After Filtration	Amount Filtered Pyrogen-free	Amount Filtered Pyrogen-positive
Control,[b] distilled water	0	...	1250	1500
A	4.8	4.6	2000	2500
B	4.7	4.9	2000	2500
C	8.0	6.8	2000	2500
D	4.6	5.0	2000	2500
E	5.1	4.7	3000	4000
F	5.7	6.8	4000	5000
G	8.0	6.5	4000	5000

[a] Plate assay.
[b] Control is distilled water containing a 1:1000 dilution of standard pyrogen.

products of manufacturers F and G were successfully filtered pyrogen-free, and it was only the fourth liter of manufacturer E and the fifth liter through the filter of manufacturers F and G that proved to contain thermogenic substances. Tests made on several lots of the same manufacturer's product gave approximately the same results and although there may be some slight differences in the adsorptive capacity of different pads, this explanation cannot be considered sufficient. It is of interest that the products of manufacturers A, B, C and D are the purest of the 7 products tested, this being based on the average units per mg. of these manufacturers' various lots of penicillin. Further, it has been demonstrated by acute toxicity tests that the lots of manufacturers E, F and G are considerably more toxic for mice than the penicillin produced by manufacturers A, B, C and D.

DISCUSSION

It is apparent from routine examination of a considerable number of ampuled preparations that in a large percentage of cases the bulk solutions filled into these ampuls were free from pyrogen but that the ampuls themselves contained on their inner surface a film of thermogenic material. Washing processes which are relied upon in the main for the cleansing of ampuls may or may not be adequate to remove such substances from the glass surfaces. In the ordinary treatment used commercially for sterilization, either autoclaving at 120° C. or dry heat at 160°–170° C. is not sufficient to destroy the pyrogenic substances which may be present. Our studies indicate that pyrogen is adsorbed on glass quite readily and that destruction of it requires heating at a temperature of at least 250° C. for thirty minutes. We have recommended that a temperature of at least 250° C. for forty minutes be used as a safety factor to eliminate pyrogens from glassware into which parenteral solutions are to be filled.[1] In carrying out tests in these laboratories to determine the presence of pyrogens we make it a practice to heat all glassware used in the test (which includes pipettes, transfer bottles, test tubes, syringes and needles) for forty minutes at 250° C. in a muffle furnace. From our studies it is apparent that pyrogen produced from different types of bacteria or from *Penicillium notatum* grown either in deep tank culture, bran or in surface cultures, is destroyed by the time and temperature noted above.

In the production of penicillin it is not possible to avoid the development at the same time of pyrogenic substances as well as other toxic substances for mice. The very nature of the process precludes the possibility of avoiding the production of pyrogen and other toxic substances since in the growth of *Penicillium notatum* there will be lysis of the mold mycelia and spores during the growth period. Such lysis releases into the surrounding medium the toxic and thermogenic substances. Practically all manufacturers of penicillin make use of adsorptive filtration, using either Seitz or Ertel filters or the equivalent, and this in itself, when properly carried out, will eliminate pyrogens from their products. Furthermore, most of the processes of extraction are such that both pyrogens and toxic substances are removed either completely or partially by the extractives used. In other words, it seems unlikely that penicillin will contain either toxic substances for mice or pyrogenic substances when properly prepared. In spite of this, an occasional lot of penicillin has been demonstrated to be thermogenic when tested in rabbits. However, in those instances where it has been possible to test a number of ampuls it has been found that only an occasional ampul contains pyrogenic material, the remainder being completely devoid of thermogenic substance as measured by the rabbit test now used for the assay of commercial penicillin. It is apparent therefore that pyrogen in "penicillin" may be largely accounted for by improper cleansing of the glassware used for filling, or failure to heat the glassware to a temperature sufficiently high to destroy this thermogenic substance.

In the removal of pyrogen from penicillin, or for that matter from any intravenous solution, by adsorptive filtration it must be kept in mind that the retentive properties of the pad used are dependent upon the concentration of the pyrogen in the solution and the size of the pad. A filter can be exhausted by overloading it with solutions containing pyrogenic material. Our studies appear to indicate that there is a direct relationship between the amount of pyrogen solution passed through an adsorptive filter and the size and/or number of pads used in the filter. It was not possible to filter 50 cc. of a 1:1000 dilution of pyrogen (which contains approximately eight times the amount of thermogenic substance necessary to produce a positive Pharmacopœial test) through a single Seitz pad and obtain a filtrate free from thermogenic substances, while two pads did remove all pyrogen from the same volume of this dilution. In order to remove the thermogenic subtance from a 1:100 dilution of our standard pyrogen it was necessary to use 5 pads in the filter, while we were unable to filter a 1:10 dilution of the standard pyrogen free from thermogenic sub-

[1] Under commercial conditions a temperature of 200° C. for two hours has been found satisfactory.

stances regardless of the number of pads. In this case, however, we were limited by the pad capacity of our filter.

It is apparent that 1250 cc. of a solution of pyrogen containing approximately eight times the amount necessary to produce a positive Pharmacopœial response in rabbits is filtered free from thermogenic substances with a 3-inch Seitz pad. Repeated experiments indicate that there is little variation in the amount of such a solution that may be filtered through a single pad of this type. However, penicillin solutions (5 units per cc.) containing the same amount of pyrogen may be filtered free from thermogenic substances in considerably larger volumes. There appears to be a marked difference between the ability of a filter pad to remove pyrogens from penicillin solutions and from distilled water containing the same amount of pyrogen and, furthermore, the penicillin produced by different manufacturers reacts differently when filtered through single 3-inch Seitz filter pads. While 1250 cc. of distilled water containing a 1:1000 dilution of pyrogen is filtered free from thermogenic substances by a single Seitz pad, as much as 4 L. of penicillin (5 units per cc.) may be filtered through a similar pad and the filtrate found free from pyrogen. In studying the relative purity of the various manufacturers' products listed in Table IV it was noted that with the purer penicillin (high unit material per mg. to which was added pyrogen) less of this solution could be filtered free from pyrogen through a single asbestos pad than could be filtered of the more impure products. It is apparent that the extraneous material in the penicillin produced with fewer units per mg. was of some assistance in the removal of pyrogens from such solutions when filtered through asbestos pads.

SUMMARY AND CONCLUSIONS

Bacterial pyrogens or pyrogens produced from *Penicillium notatum* may be destroyed by a temperature of 250° C. for thirty minutes.

All glassware used in tests to determine the presence of pyrogens in solutions, including pipettes, transfer bottles, test tubes, syringes and needles, should be heated at a temperature of 250° C. for at least forty minutes before use.

In the process of producing penicillin both toxic substances for mice and thermogenic substances are also produced.

Pyrogens in penicillin solutions may be removed by adsorptive filtration with asbestos pads.

There is a relationship between the volume of penicillin which may be filtered free from pyrogen through a given filter unit and the purity of the product.

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WANTED—1944 PROCEEDINGS ISSUE

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A System for the Aseptic Processing of Ampuls*

By John H. Brewer and Fitzgerald Dunning

The processing of biologicals and other preparations which must be filled into containers with small openings presents many difficulties which cannot be readily overcome using the usual pharmaceutical equipment. Up to the present there has not been any general line of equipment especially designed for the washing, filling and sealing of ampul and vial preparations which did not involve the individual handling of ampuls or vials. The system to be described herein has many advantages over usual practices and the equipment utilized is economical in cost, time and space required.

This system was originally developed in the pharmaceutical laboratories of Hynson, Westcott & Dunning, Inc., for the filling of aseptic preparations, but has since found widespread use in many other industries where the necessity for filling solutions into small containers has arisen. The greatest single advantage of the technique employed lies in the fact that it is not necessary to handle ampuls singly in any of the steps involved in the process. Ampuls are received from the manufacturer and are assembled in racks in copper boxes by hand, but subsequently, throughout the washing, drying, sterilizing, filling and sealing operations, no individual ampul is touched. It is obvious that asepsis is more easily maintained by this procedure, utilizing our specially designed equipment.

To outline more clearly the technique of the entire aseptic process, it will be necessary to consider each operation separately. Four individual operations, all involving the use of specially designed equipment, are required, namely: (*a*) washing and drying of the ampuls or vials; (*b*) sterilization of the ampuls; (*c*) use of aseptic rooms; (*d*) aseptic filling and sealing process.

Washing.—Because of subsequent handling and storage, it is practically impossible for glass manufacturers to supply clean ampuls, which fact makes necessary a satisfactory washing process. Heretofore the washing of ampuls and vials has been difficult and uneconomical because of the lack of a convenient method of handling large numbers of small, fragile containers by machine operation. The design of a small-mouth vial or ampul, unfortunately, precludes the washing of these containers by any easy method, such as simple rinsing. The smaller sizes, 1 cc., 2 cc. and 3 cc., cannot be filled by submerging, for the air is trapped in each ampul. When small ampuls are filled by an enforced method, it is then necessary to shake out the water or blow it out by means of an air jet. The usual practice is to place the ampuls on capillary pipes and then blow through various combinations of air, steam, detergent solutions or, perhaps, just water and air. By such methods, however, certain contaminating factors cannot be removed and this led to the development of a washing technique which has proved effective.

Fig. 1.—Ampul washing machine showing cover open and admission of water with ampuls in position for filling.

This technique is based on observations indicating that 1-cc. ampuls with constricted necks could readily be filled in a few seconds by centrifugal force. In addition, further observation revealed the fact that the same ampuls could be emptied by centrifugal force by simply reversing them in the centrifuge tube. Not only did the ampuls empty, but every droplet of water was removed. In subsequent oven drying, therefore, no material which may have been dissolved in the

* Received Dec. 23, 1944, from the laboratories of Hynson, Westcott & Dunning, Inc., Baltimore, Md.

Fig. 2.—General view, showing dust-free rooms and equipment.

Fig. 3.—Filling of ampuls under glass shield. Note that only those ampuls actually being filled are exposed.

rinse water could be left as a deposit on the glass and thereby serve to contaminate the solution later filled into the vial.

Utilizing this principle, a machine was constructed to hold twelve gross of 1-cc. ampuls (Fig. 1). Using it, one operator can wash, rinse and dry these ampuls in fifteen minutes, making the hourly output of the machine over six thousand ampuls. Not only are the ampuls washed on the inside, but the outside as well, which, of course, is not true in the jet type washer. The copper boxes and racks containing the ampuls also are given a thorough washing. The same washing machine can be used for various size ampuls. The larger the ampul, the fewer it is possible to wash in a given time. Machines of varying capacity embodying this principle are now commercially available.

To illustrate graphically the method of washing: Ampuls as received from the manufacturer are placed in racks, each of which contains a row of 24 ampuls (1 cc.). Twelve such racks are placed in a copper box and held in place with a snap-on retaining grill. Six such copper boxes are placed in the centrifugal washer. Hot water is run in and a suitable readily soluble detergent is added. The machine is run for twenty seconds, during which time all ampuls are completely filled. On stopping, the drain valve is opened and the detergent solution run into a waste line. The boxes are then reversed and the machine run for one minute, by which operation the detergent solution is evacuated. The ampuls must be rinsed by repeating this process using clean, hot water. The boxes are withdrawn from the washer, the retaining grill snapped off, and the copper box closed with a close-fitting lid.

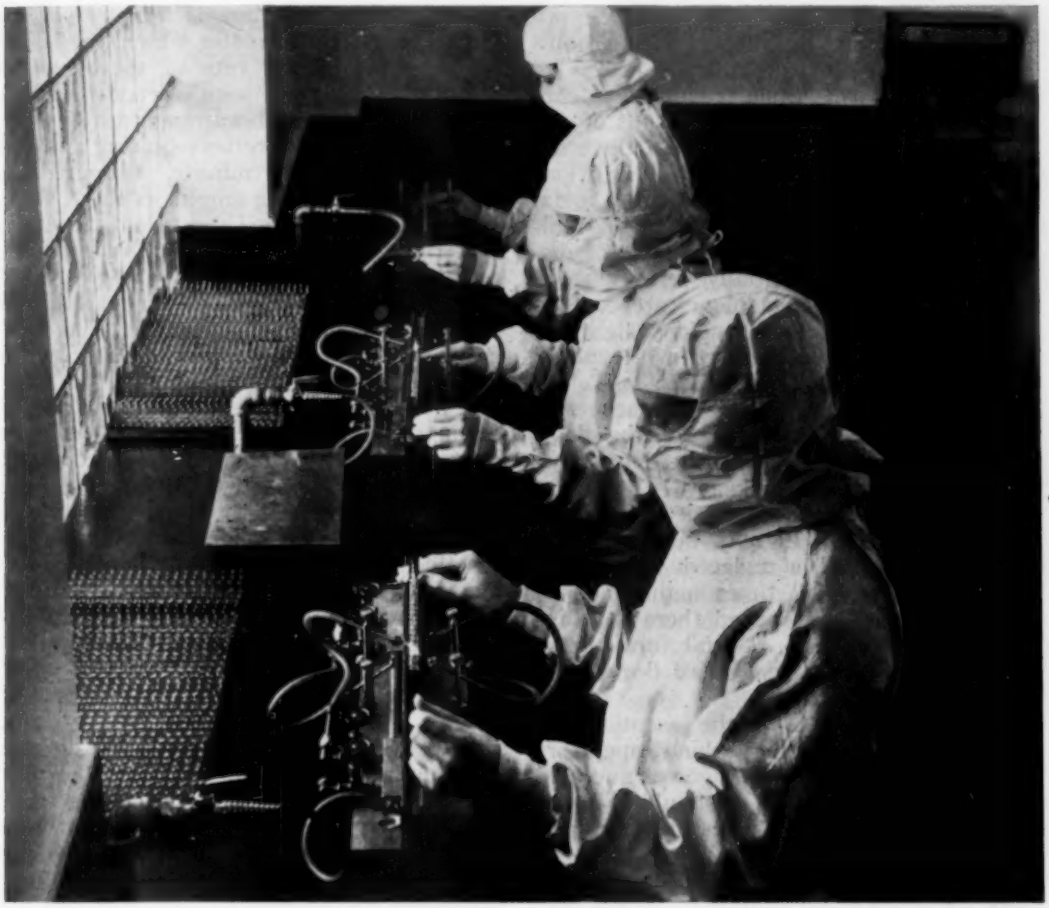

Fig. 4.—Ampul sealing operation. Sealed ampuls are in rack against window. Unsealed ampuls are kept under cover in box.

The equipment required for this washing process occupies a floor space of approximately 9 square feet, far less than that usually required. The time and labor involved is only a fraction of that ordinarily consumed. For example, one operator can

wash and dry by this process approximately 48,000 1-cc. ampules in an eight-hour day.

The Sterilizing Process.—The closed boxes, following removal from the washer, are conveyed on a hospital cart to the sterilizing oven. Since this is dry glassware, the sterilization is carried out in a hot-air sterilizing oven at a temperature which will both sterilize and destroy pyrogens.

The Aseptic Room for Filling and Sealing.—These rooms (Fig. 2) are especially designed incorporating many of the known features utilized in hospital operating rooms and in bacteriological transfer rooms to promote asepsis. Such rooms are constructed with smooth-surfaced walls of any suitable building material so that there are as few joints as possible. If glass partitions are used, the glass should be set in a metal angle inside of the usual ridged sash. Ceilings are sloped, permitting the use of disinfecting sprays to initially remove dust. All corners are rounded, floors are covered with linoleum, ledges are avoided, light fixtures are recessed and fitted with rubber gaskets to prevent "breathing." Tables are built out from the walls on brackets so as to permit easy cleansing of the floor. Outside windows are constructed of thoroughly caulked glass bricks.

The rooms are air conditioned in a manner so that air filters may be readily removed and cleaned or replaced. A positive air pressure is at all times maintained so that when doors are opened there is no inrush of unfiltered air. Metal furniture is used so that it may be washed down with disinfecting solution.

Entrance is made to the aseptic area through an air-lock in which operators change to sterile masks and gowns and also to irradiated work shoes. It should be noted that the rooms and air-locks are equipped with steri-lamps.

The Aseptic Filling and Sealing Operations.—Copper boxes containing sterile ampules are transferred through the air-lock into the aseptic room. One such box is placed in a specially designed filling track which keeps the ampuls under cover except when each row is successively exposed under the lighted filling slot (see Fig. 3). The operator moves successive rows of ampuls to the filling slot by means of a knob operated with the left hand. The solution is introduced into the ampuls by an automatic pipetting machine. This automatic pipetting machine, which was originally designed for measuring out reagents for the Wassermann test, was developed in the Johns Hopkins Hospital, in 1938, and makes use of standard ground-glass syringes and has an unusual accuracy. The glass syringe and valve mechanism is so arranged that the entire assembly can be sterilized with all necessary connections to bulk containers and is very simply attached to the machine by means of two knurled thumbscrews. Many of these machines are in use measuring amounts as low as five-hundredths of a cubic centimeter, and with proper selection of syringes and delivery tips, volumes ranging from 0.05 cc. to 60 cc. can be accurately dispensed. The machine is equipped with a governor-controlled motor which can be set at a predetermined rate of speed, which speed does not vary with electrical current fluctuation. Some laboratories are now using this machine for deliveries requiring as much as 120 strokes per minute, although the usual speed for filling ampuls is about 10 to 40 strokes per minute, dependent upon the material and volume to be filled into the ampuls. This filling operation is protected from contamination by a sloping glass shield extending beyond the edge of the table.

After each box of ampuls is filled, it is passed on to the sealing operators (Fig. 4). The box lid is slid back to expose one rack of ampuls at a time. Each rack is successively removed from the box with sterile forceps. The ampuls are sealed by sliding the rack through flames from burners fed by carburetted gas. The sealer contains an adjustable slot which will accommodate various sizes of ampuls by the simple adjustment of two thumbscrews. The angle of the flames striking the neck of the ampul is approximately 15°.

The time required for sealing one rack of ampuls (24) is approximately fifty seconds. Therefore, the length of time of exposure for any one ampul would be only the few seconds in which it is under the filling track and the fifty seconds in which it might be in the sealing machine. Since these operations are carried out under glass shields in dust-free rooms, the chances of contamination are very low.

This efficient and economical system of filling and sealing ampuls as described above has been in operation in our plant for about four years. The satisfactory results have led to its adoption by many processors of ampuls and small vials. Our staff collaborated with several producers of penicillin, offering full information and assistance so that the system might be adapted to the packaging of that important drug. It has been this rather general interest which has led us to write this descriptive article. Plans for the establishment of the system and equipment are being offered commercially now by the Baltimore Biological Laboratory.

Needed Medical Research Developments in Industry*

By Austin E. Smith†

Recognizing the need for reviewing the present status of medical research and voluntarily undertaking a program to meet the needs for continuous progress will result in continued freedom in research and benefits for all participants. To do otherwise will cause restrictions over research programs and probably recession of our present speed of progress. Thus, plans for postwar research and medical improvements should be made now.

Competition between commercial organizations is always keen, and the drug industry provides no exception. Many of the smaller organizations are now in a position to challenge the larger companies in new developments, but their continued success, like that of their competitors, will depend on research development, organization and promotion, and also on the activities of other organizations and individuals who are interested in medical research. About one year ago I plotted, with the aid of financial reports, catalogue and confidentially submitted information, the net sales, profits, research investments, changes in production lines and number of employees of several large firms over a ten-year period. It was readily apparent that finanical success and growth of a firm bear a direct relation to research development and promotion; the firms most active in these three fields were making the soundest gains.

What, then, are some needed research developments?

TRENDS AND RECOGNITION

Funds for Research.—One thing that can and should be done by busines is to return more money to research; at least twice or three times as much as is now expended should be turned over immediately to the development of new manufacturing technics, new products and new methods of treatment. Greater amounts at a later date are not inconceivable. The funds should be divided for short-term projects and long-term projects, the division being made according to the needs of the firm and its plans for future survival and expansion. There are some houses which have not contributed sufficient original material to gain even a smattering of a scientific reputation and these firms should be especially concerned with long-range viewpoints. One argument for more interest by manufacturers in long-term studies is that there is no better salesman than good publicity, and favorable publicity is bound to result if a manufacturer makes an outstanding contribution to the conquest of world-wide killers such as cancer, tuberculosis and hypertension.

INTRA-ORGANIZATION RESEARCH

Planning a Research Program.—In preparing a research program, consideration should be given to the facilities, including personnel and equipment, which are available in the home organization and what can be done with these facilities so that most can be obtained with the least expenditure of men and material; the establishment of clinical relations to support laboratory finds and determination of the ultimate extent of the claims that can be made; the establishment of relations with institutions such as universities and research centers; and even the establishment of relations with other manufacturers so that unnecessary and expensive duplication of research may be minimized.

Research Personnel.—The organization of a research staff depends on obtaining men and women with sound training, scientific education, intellectual honesty, ambition, respect for fellow men imagination, to name only the most important requirements. It is unwise to take makeshifts simply because good men are a little more difficult to obtain. It will be worth while in the long run to get the better men and give them salaries and opportunities such as freedom of thought and bonuses for ideas, so that they will be content to stay and give of their best. Two of the most important items in the young scientist's life are the opportunity to undertake good research and the prospect of family security. The organization must be adequately trained to pull together. All departments must work harmoniously and have their activities correlated under good leadership to rule out conflicts, petty jealousies and inefficiency.

Cooperation of Business and Research.—In industrial organizations business and research must jointly make suggestions and consider the other's viewpoint with interest

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† Secretary, Council on Pharmacy and Chemistry, American Medical Association.

and appreciation. There must be mutual cooperation and respect. Many good researchers are leaving industry to return to academic life and to go elsewhere. Some of these men are accepting smaller salaries, a fact which must imply considerable discontent with present arrangements. A few of the men do not like their employers' promotional policies; others resent the lack of interest in research which shows no immediate possibilities for finanical gain; some are leaving because they were not permitted to finish projects which they had started independently or even at the firm's suggestion. When these men are given no opportunity for original investigation, their natural curiosity becomes dulled and their scientific attitude regresses. Under such conditions good men will move on to other jobs. This present outflow of well-trained men from industry seems to be almost as great as the inflow of a few years ago.

Even though the present day is one in which there is much change in all walks of life, there can be no escaping the fact that the men in the drug industry are especially trained and are especially capable of making mature decisions. Their training and experience have been such as to allow them to develop considerable leadership and constructive thinking; there must be good reason when such men express dissatisfaction and are willing to sacrifice income and, in some instances, security so that they may pursue their chosen work elsewhere. Now is the time to determine the cause of this dissatisfaction and rectify it, as these men actually represent the building stones of the foundation on which pharmaceutical houses rest. Every worth-while scientist merely asks a chance to prove his value and to participate in good, sound investigation.

Utilizing the Results of Research.—Manufacturers whose researchers have produced interesting preparations should determine the extent of their possibilities and not just leave them idle on the shelves. Too often, hardworking researchers have devoted endless hours to the study of new drugs of promise only to see them ignored by the dominant executives. Each group that has been responsible for the development of a new agent should be permitted to sit with the other groups of the organization and thrash out what is needed in the way of further study, what changes are necessary, what present features should be worked over, and what future plans—promotional and otherwise—may be made. In this way a composite picture is obtained which may prevent errors, perhaps may even save lives. No one group should be permitted to dominate another purely on business reasons; sound scientific reasoning will provide the best results and it can be balanced with good business practice. A director of research should be permitted to devote a fair share of his time to general research projects of his own interest or of prime concern to humanitarian interests and should not be expected to devote all his time to problems of purely financial concern unless these represent emergencies. Facts known by one group should not be hidden from others who work cooperatively. This is true not only of groups allied in the same parent organization, but of groups working without the organization; for example, research clinicians. On the other hand, those individuals who are devoting their time freely to the development of a new drug should not be constantly hounded by members of the business department and others who are primarily interested in immediate financial possibilities. As results are obtained, they will become known and, if good, the firm will benefit in due time.

ADDITIONAL ACTIVITIES

Research and the Pharmaceutical Manufacturer.—There are a few aspects of research which are worthy of particular attention by the pharmaceutical manufacturer. For research there are needed men, facilities for their work, adequate remuneration, and worth-while problems to attack. Modern science has of necessity become divided into many branches and to master any one branch requires as many years of a young man's life as he cares to spend before beginning to earn his own living and assuming the responsibilities of life. Nevertheless, achievements can best be made possible by men who are masters of two or more branches of science and who can visualize the application of discoveries, technics and achievements in one science to another. There are needed a number of key men of this type and the more the better. For example, there are needed men who have mastered medicine and physics, medicine and physical chemistry or organic chemistry or biochemistry, medicine and psychiatry, and medicine and various branches of biology.

The value of pharmacists who have had extensive teaching and research experience and postgraduate education should not be overlooked. Specially trained men are in a position to see how best to apply or investigate the principles of that branch of science in relation to medicine, and they can then undertake the responsibility of having under

their direction others who have perhaps mastered only one branch but who, nevertheless, would be of great aid in initiating and conducting research and determining the significance of the work. To master medicine and one other branch of science requires from ten to twelve years of university training; there should be some arrangement by fellowships or otherwise whereby such men as they grow older, coming up through the twenties can feel self-supporting and even, if they wish, get married. Pharmaceutical houses can contribute much on this score, as some already have, and can contribute even more when men return from the wars to continue their training. Actually, this is a form of research and the dividends are good.

Until the last decade or two, research has been done mainly in the universities but now, especially in the pharmaceutical industry, much of it is being done in commercial establishments. In commerce stress is of necessity placed upon what is called "practical research" yet much, perhaps most, of the practical applications of science today are based upon experiments which when first done were not thought "practical" in any sense of the word. Workers in medical research have always had their eyes turned toward the practical application of their work. This is only natural because medicine is a practical science in contrast to more fundamental sciences such as physics and biology.

Fundamental Research.—However, to provide the best over-all picture, some men in medical research must forget the practical side now and then and do research just for the pleasure and curiosity involved. Perhaps much of this type of research may never have a practical type of application, but some of it is bound to lead to new concepts of human physiology and pathology and the correction of pathologic conditions. This nonpractical type of research can be done well in the universities where those involved are not concerned with making money out of the research, although, of course, laboratories have now been equipped in many pharmaceutical houses where such work can adequately be undertaken on home grounds. To maintain this so-called "fundamental research" in all departments of medical schools, more funds should be made available with no strings attached from individuals and from commercial establishments. All too often today men doing research in medical schools are forced to direct their research upon practical problems in order to get financial assistance from outside sources.

Simplifying the Review of Research Literature.—Another type of development that is needed concerns reviews of work already done. The volume of reports turned out today and in the past two decades is such that no one person can be expected to know it all. To a person beginning research in a field unknown to him, the review of literature is now a formidable problem which may consume a large part of the time which he has at his disposal; and at the same time different persons in different laboratories working upon the same problem or in the same field may be spending as much time reviewing the literature and making notes upon the very same articles. This is a waste of time and, more important, it cannot be done thoroughly by all workers. To overcome this, more monographs should be prepared and written in such a way that the one small field which is selected is covered exhaustively and completely up to the date of publication. Such monographs would have a small volume of sale and their publication would have to be subsidized so that each interested center, including medical schools, could afford to purchase a copy of each monograph. Every few years—say four or five—the monographs could be brought up to date by succeeding volumes. Three types of books are needed in the different branches of medicine: a textbook covering the essentials, a group of reviews covering in more detail broad subdivisions of the subject in question for the use of specialists in keeping up with the whole subject, and a series of monographs upon very small fields which are covered completely for the use of the research men in these fields. The last type of book is the hardest to write because it involves reading all reports and an intelligent sifting out and interpretation based upon experience. For this reason it can probably be done best by older men, and in order for them to do this they must be relieved of many of their administrative duties so they may devote more time and thought to the subject.

COUNCIL CONTRIBUTIONS

Research developments in the medical field require the attention of all interested parties if the best results are to be obtained. The Council on Pharmacy and Chemistry has followed with intense interest the use of drugs for the past forty years; it has prepared reports on therapeutic advances; it has initiated research on special problems; it has provided many thousands of dollars to help defray expenses in research projects. Now it is prepared to go farther.

Sources of Drug Samples.—Through the offices of the United States Pharmacopœia Revision Committee and the League of Nations Health Organization samples of certain well-known substances may be obtained for examination and standardization. But there is no office through which samples of nonofficial drugs may be obtained. To meet this need the Chemical Laboratory, which examines all preparations accepted by the Council for inclusion in New and Non-official Remedies, is prepared to undertake the proper storage of sufficient amounts of N. N. R. drugs to meet requests for specimens for examination. The mechanics for this plan are now being prepared; extent of activity will be determined partly by the nature of the requests received at the Council office and the Laboratory.

The Council and Clinical Evaluation of New Drugs.—And last, but certainly not least, is the Council's willingness to participate in the clinical evaluation of new drugs of significant promise. Until now, with the exception of aiding therapeutic research by grants and reports, the Council has usually considered drugs only after the evidence has been accumulated; it has not initiated clinical trials on substances fresh from the laboratory. However, its members have participated in the activities of the Office of Scientific Research and Development and National Research Council, and it believes that the plan of cooperative clinical research which was instituted during this war is extremely valuable. It also believes the British Therapeutic Trials Committee has proved its usefulness. And finally, the Council believes that the American Medical Association should use its facilities and contacts toward furthering such work, if it is necessary to supplement or continue the activities of the O. S. R. D. After consideration, the Council formed a special committee known as the Therapeutic Trials Committee to deal with the subject.

The Therapeutic Trials Committee of the Council on Pharmacy and Chemistry believes that the activities of such a group at the present time should take the form of mediating between manufacturers and clinics; that the work should be confined to new products of definite and outstanding promise, on which adequate laboratory work is available; that the Committee should have full jurisdiction in the choice of projects that it would sponsor; that the costs incurred in the actual trials should be the subject of contract between the manufacturer and the clinic conducting the trials, but that the overhead administrative expenses would be a legitimate and useful call on the Association; that plans should be developed, but that they need not be put into full effect until the proposed peacetime plans of the O. S. R. D. have crystallized. Such plans already have been developed and considered by the Council.

CONCLUSION

One of the most effective ways of maintaining free enterprise and continuous progress is through unrestricted research. In problems affecting health such progress is essential and, in effect, becomes the moral responsibility of all interested parties. If research is properly conducted, the results will be fruitful—an important factor to weigh upon public opinion when thought is given as to who shall control research, those who have been making a study of it for a lifetime, or those who previously have had no intimate acquaintance with the subject, but become entitled by law to engulf activities to satisfy personal greed. Now is the time for all individuals interested in free enterprise and medical progress to put into effect plans for creating improvement. To delay unnecessarily will result in personal loss, perhaps even of freedom of research activity.

COMMUNICATION TO THE EDITOR

Is the Indiscriminate Use of Penicillin Imminent?

To the Editor:

Penicillin will soon be available for general civilian use. It is understood that commercial firms will soon have sufficient supplies to market it through their usual channels and that it will soon reach the local drugstore. There it will almost certainly be available to the general practitioner on much the same basis as any other biological. While still expensive, its price will not be prohibitive. Its use will, in all likelihood, be widespread, both because of its remarkable efficacy and because of the magic of its name to doctor and public alike. Further, the recent announcement by Libby (1) that

penicillin in cottonseed oil solution enclosed in gelatin capsules has been satisfactorily administered by mouth to human beings appears to herald even more extensive use of penicillin and its related compounds.

It would seem that now is the time to take proper safeguards against their indiscriminate use. The proper patterns, set now, might well avoid the tragedies that accompanied the introduction of the sulfa drugs and the waste that accompanied the introduction of purified vitamin preparations.

Time alone will tell what the abuses of penicillin are to be. Its parenteral administration has resulted in an infrequent but definite number of reactions such as urticaria, and fever, reported by the Committee on Chemotherapeutic and Other Agents of the National Research Council (2, 3), thrombophlebitis, and even contact dermatitis (4). Penicillin may be harmful in acute rheumatic fever (5), and penicillin-resistant strains may develop (6). It is not yet fully known how many of the reactions described above were due to impurities in the penicillin preparations then in use, nor is there much information about the toxic reactions, or lack of them, with penicillin taken *per os* in a vehicle suitable for its ultimate absorption. Penicillin so far appears to be a far safer drug than the sulfas, and its indiscriminate use might actually be accompanied by fewer reactions than occur now with aspirin, or by less economic waste than now accompanies the indiscriminate use of purified vitamins.

Perhaps the greatest danger from the indiscriminate use of penicillin is of another sort: namely, the development of a sense of false security. Penicillin is of no use in many diseases, and in others there is danger from inadequate dosage. For example, it is not hard to visualize that a patient with a chancre would hear that penicillin cures syphilis, would procure some, take it (or have it administered) in inadequate dosage, see his chancre disappear, feel that he has been cured of the disease, and then develop neuro- or cardiovascular syphilis many years hence. Numerous other examples can be imagined.

Nor does it appear unlikely that a period of commercial exploitation of penicillin will soon set in. It is not at all unlikely that the patent medicine manufacturers will soon obtain a supply of the drug and that soon we may have intense salesmanship of penicillin cough drops, and penicillin mouth wash, not to mention penicillin skin cream or even penicillin laxatives. Perhaps such wild unleashings of the imagination will prove to be too conservative.

Certainly it is too early to know yet just how the matter will develop. It would appear to be none too early, however, to reassure ourselves of at least the following:

1. That adequate research will be undertaken, and maintained, to ascertain the best forms of penicillin and related compounds for therapeutic use, their indications and contra-indications, their best routes of administration, and their toxic reactions;
2. That adequate education of the doctor, the druggist, and the public be carried on to assure the proper use (and restraint from use) of penicillin;
3. That proper steps be taken by professional organizations, particularly the medical and pharmaceutical associations, to prevent possible abuses of penicillin and to assure its availability where needed;
4. That the drug industry take the necessary steps to foresee and prevent, mainly through voluntary discipline, possible abuses and maldistribution;
5. That the Government not relax its controls until there is evidence that to do so would not lead to shortages in cases where penicillin is necessary, or contrariwise, to a riot of commercialized penicillinization of our population.

Some of the emphases in this communication may be mistaken, especially since I am no longer associated with work on penicillin. [My former contact was outlined in a previous communication (7).] There are quite clearly dangers from the failure to use penicillin in diseases which it alone will cure. There are also dangers of too stringent controls on its use. These are not discussed here since the opposite factors appear to need more emphasis now.

It would appear that full discussion of this question, perhaps mainly in the columns of THIS JOURNAL, would be of considerable importance in avoiding mistakes which might prove tragic as penicillin becomes more readily available. Under the guidance of the Food and Drug Administration, the Office of Scientific Research and Development, the National Research Council and the War Production Board, a good start has been provided for penicillin. It would be sad if its future were less wholesome.

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(3) *Ibid.*, **123**, 100(1943).
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(5) *Ibid.*, **126**, 282(1944).
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(7) *Ibid.*, **124**, 1219(1944).

Book Reviews

Bacteriology and Allied Subjects, by LOUIS GERSHENFELD. Mack Publishing Company, Easton, Pa., 1945. xxiii + 561 pp., 20 illus. 17 x 24.75 cm. Price, $6.00.

Dr. Gershenfeld has written into this book an interesting, instructive, and thought-provoking historical introduction which should captivate the interest of any reader. The chronological listing of the important dates and discoveries in bacteriology alone justifies its inclusion on any scientist's book shelf. The chapter on activities and classification of bacteria is also worthy of mention, especially for beginners, for it presents facts and terminology necessary to the subject of bacteriology in an interesting fashion well supplemented with explanatory notes. Apparatus and its uses are similarly treated, even to stains, formulas for their preparation and use, and formulas for the preparation, storage, and use of culture media.

The common pathogenic and nonpathogenic organisms are discussed with specific information given for each which is of prime interest to the average student. These are considered under headings of Cocci, Bacilli, Bacilli (spore forming), Spirilla, Spirochetes and allied organisms. Colored plates of stained organisms are included to good advantage. More of these illustrations could probably have been included to good advantage for the laboratory worker. The discussion material for the organisms is clear and concise, representing rather an improvement over many of our present crop of bacteriology texts and references. It covers adequately their classification, morphology, measurements, staining behavior, tests for identity, special reactions, and related organisms. These subjects are well done.

The special treatment of the Fungi is good in clarifying the confusion which generally exists in the student's mind. Their growth habits, means of reproduction, and clinical significance are noted. These and the yeasts are deserving of more attention than they have heretofore received for the most part. Although the molds such as Penicillium are mentioned, the reviewer believes more detail could have been devoted to these antibiotic substances. A fine chapter is devoted to the organisms of commerce and industry which is appropriate in acquainting the reader with the wide use industry makes of bacteria and yeasts. Too frequently inattention to this phase of bacteriology leaves the reader wrongly impressed that all organisms are dangerous pathogens. Useful species should receive due recognition as they have in this text.

Methods for the control of bacteria are as essential to the bacteriologist as the organisms themselves. Dr. Gershenfeld has recognized this fact by devoting more than passing notice to the actual methods and agents of sterilization. Physical agents, ultraviolet light, filtration, heat, and the various chemical agents used are discussed in enough detail to be of value to the laboratory worker. The lecturer in pharmacology and materia medica will find this portion of the book of interest. The inclusion within its scope of the arsenicals, sulfonamides, and dyes, as well as penicillin and gramicidin, makes the book a good source of information on antisepsis. Sterile solutions are becoming an important part of the instructional program in our colleges of pharmacy; consequently additional attention will have to be directed to their preparation and use. A table of sterilization procedures recommended for various parenteral solutions and suspensions is included.

The author has wisely devoted a liberal amount of discussion to the topic of animal parasitology which will be welcomed by the teachers of biological sciences. The lecturer in materia medica and pharmacology as well as the bacteriologist and parasitologist will find a wealth of readily usable information here to augment his lectures on anthelmintics. The more common types of parasites are all included, together with the information pertinent to their propagation, abode in the body, and general characteristics. This is a timely inclusion in a text of this nature, for with the return of our service men from all over the world we are certain to give more attention to parasitology. This is supplemented with an excellent part on insect control, insecticides, and fumigation which has too long been neglected in our pharmaceutical curricula.

In keeping with the emphasis being placed on immunity and biological products, the author has devoted a good part in the book to a clear, concise discussion of such topics as antibodies, immunity theories, allergy, and their relationship to disease. Especially good are the chapters on biological products proper, including an introduction to biologicals and covering the subjects of antitoxins, with specific reference to those official; the antivenins; serums; antigen preparations such as bacterial and virus vaccines, toxins and toxoids; diagnostic tests; tuberculins; and biological tests as aids in diagnosis. The concluding chapters on methods of examination for air, water, milk and dairy products and other foods, together with detailed information on the collection and bacteriological examination of clinical specimens are well done, even to mentioning methods for testing and evaluating the efficiency of disinfectants. Suffice it to say that the numerous and diverse subjects covered by the author in this book are well done, especially for students and teachers limited in the time allotted to them by our average pharmaceutical curricula.

The value of the book is further enhanced by being well documented and provided with an unusually complete index. The printing, binding, and paper are of the same excellent quality found in other books produced by the Mack Publishing Company.—L. DAVID HINER.

Vitamins and Hormones, Volume II, edited by ROBERT S. HARRIS and KENNETH V. THIMANN. Academic Press, Inc., New York, 1944. xv + 514 pp. 15 x 23 cm. Price, $6.80.

This is the second edition of an annual review in the vitamin and hormone field (see THIS JOURNAL, **33**, 31(1944). As in the first of the series, each section is written by an expert in the particular field.

In this edition the following subjects are discussed: the role of vitamins in the anabolism of fats, the chemistry of biotin, the nutritional requirements of primates other than man, physiological action of vitamin E and its homologues, the chemistry and physiology of vitamin A, para-amino benzoic acid—experimental and clinical studies, a critique of the etiology of dental caries, vitamins and cancer, effect of androgens and estrogens on birds, hormones in cancer, and x-ray crystallography and sterol structure.

The reviews follow the same general pattern as in the previous edition and show the same editorial care. Pharmaceutically trained people should be interested in this series.—M. W. GREEN.